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The influence of experimentally injected autogenous blood in diarthrodial joints in the equine.

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THE INFLUENCE OF EXPERIMENTALLY INJECTED AUTOGENOUS BLOOD IN DIARTHRODIAL JOINTS IN THE EQUINE

by

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INTRODUCTION

Joint trauma with subsequent hemorrhage into the joint cavity is not uncommon in the equine. It has been suggested[1] that the cartilaginous lesions which may follow are similar to those that have been described as degenerative osteoarthritis. These lesions appear to occur more frequently in horses having a history of more than one traumatic episode. In these animals, the joint capsule is often distended with a sero-sanguinous or hemorrhagic synovial fluid.

These observations and recent literature on the hemarthroses, posed the questions as to whether or not hemorrhage into the joint cavity might initiate articular disease and could be involved in the pathogenesis of degenerative osteoarthritis.

The answers to these questions would be of practical value to the clinician when considering therapeutic and surgical techniques. Radical joint surgery and occasionally arthrocentesis produce intraarticular hemorrhage. Therefore, more discrimination may prove to be indicated when using these procedures. In addition, such knowledge would be of value when considering a course of treatment of hemarthrosis induced by

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[1]E. D. Roberts, Assistant Professor, Department of Veterinary Pathology. Iowa State University of Science and Technology, Ames, Iowa. Necropsy evaluation of arthritic joints. Private communication. 1963.

nonsurgical trauma.

Consequently it seemed essential that the effects of autogenous blood in the equine diarthrodial joint be determined.

REVIEW OF THE LITERATURE

Villonodular Synovitis

Lesions of the synovial membrane subsequent to intraarticular hemorrhage have been reported in man. However, the effects of hemorrhage upon the cartilage needs further study.

Key (1929) reported his observations on the exudates and descriptions of the histologic changes in the joints of normal rabbits following the injection of citrated homologous blood and India ink (carbon particles).

The alterations in the synovial membrane cells were confined to those cells supported by loose subsynovial tissue. The nuclei became larger, more spherical in outline, and more reticular in structure. The cells became larger and in some instances developed processes resembling those of fibroblasts. These events started on the first day, reached their maximum on the fourth or fifth day and returned to near normal by the 11th or 12th day. Leukocytes and macrophages were seen in the subsynovial tissue. Large irregular macrophages had engulfed one or more erythrocytes within four or five days postinjection.

Key stated that the greater part of the blood cells probably escaped by passing between the synovial cells and back into the tissues of the synovial membrane and joint capsule. This was accomplished either by phagocytosis or infiltration into these tissues as free erythrocytes. He also stated that erythrocytes were sometimes incorporated into

small clots which became adherent to the synovial surface.

Another group of rabbits was injected intraarticularly seven times over a period of 24 days. The joints were then studied as in the preceding experiment; one joint being examined each day for 12 days after the last injection.

In the latter experiment, the synovial membrane was very thick after repeated intraarticular injections of blood. The synovial lining cells were increased in size and number, but the thickening was the result of the proliferation of connective tissue and infiltration by macrophages and leukocytes. On the third day after the last injection, the leukocytes had largely disappeared from the joint tissues and the macrophages had increased in number at this time. In the joints examined 11 and 12 days after injection, there was a gradual reversion of the tissues to a condition approaching normal.

Along the borders of the hyaline and fibrocartilage, the synovial membrane was, in many places, hypertrophied to form fringes or villi which tended to project over the adjacent border of the cartilage. The surface of the cartilage was usually normal, but in some areas it was roughened and frayed. The cartilage cells were not enlarged and showed no proliferation except near the borders where the reaction in the synovial membrane tended to involve the adjacent cartilage for a short distance. In these areas, there was some infiltration of the cartilage with leukocytes and macrophages, and some of the

peripheral cartilage cells were enlarged and resembled fibroblasts.

Many authors prior to 1941 had described nodular or tumor-like lesions of the synovial membranes of joints, tendon sheaths and bursae. The tenosynovial lesions were variably called xanthomas, xanthogranulomas, giant cell tumors or myeloplaxomas of the tendon sheath. The synovial and bursal lesions were classified in a similar manner, but in addition certain forms were referred to as chronic hemorrhagic synovitis, giant cell fibrohemangiomas, fibrohemosideric sarcomas, sarcoma fusogigantocellulare, and benign or malignant polymorphocellular tumors of the synovial membrane.

Galloway et al. (1940) published their findings on this type of lesion in man. They referred to it as a xanthoma. Grossly the synovial membrane was lobulated, "encapsulated", firm, and greyish-yellow to reddish-brown. Microscopically, foam cells, foreign-body giant cells, hematogenous pigments and fat were nearly always found and were characteristic features. Cellular and fibrous elements were always the same but in some areas the fibrous tissues predominated, while in others, the cellular elements were foremost.

They stated that the primary etiological factor was a pre-existing alteration of "lipoid" metabolism. Secondarily, either trauma or infection occurred. This was accompanied by minute hemorrhages which allowed lipoids and pigment to be

deposited by the circulation. These changes developed slowly or rapidly, depending on the severity of the hemorrhage and the subsequent release of lipoids and pigment. As the process progressed, vascularity increased and more hemorrhage occurred.

Jaffe et al. (1941) stated their concept of the connection between the above listed conditions and pigmented villonodular synovitis.

Villonodular synovitis occurred in a diffuse or circumscribed form. In the diffuse form, the membrane appeared brownish in color and was covered by villous and/or coarse nodular outgrowths. The circumscribed form was usually a solitary nodule. The cytological pattern was usually one in which a very cellular area, composed of closely compacted polyhedral cells and variable numbers of multinuclear giant cells and "lipoid" (foam) and hemosideric cells, alternated with areas in which intercellular collagen and hyaline largely crowded out the cells and dominated the picture. The supporting stroma contained many blood vessels and much blood pigment. Most synovial lining cells also contained a considerable amount of hemosiderin in their cytoplasm. Occasionally a vessel was cuffed by lymphocyte-like cells but more often by pigment-bearing cells. Grossly the areas that appeared as matted villi were fused. Intergrowth and compaction of villi or cross strands of tissue between villi often consisted merely of two opposing layers of synovial cells which formed isolated

spaces of joint cavity.

They developed the concept that xanthomas, giant cell tumors, and other similar conditions of the synovial membrane were various stages in the same process which they referred to as villonodular or pigmented villonodular synovitis. Their terminology has been generally accepted.

Jaffe et al. (1941) also stated that the lesion was definitely inflammatory in nature but did not think that the etiology was simply intraarticular hemorrhage or a disturbance of "lipoid" metabolism. They cited an experiment in which autogenous blood was injected repeatedly into the femoro-tibial joints of rabbits. The conclusion was that blood induced little permanent damage to the synovial membranes. Jaffe (1958) has maintained his views in regard to the role of hemorrhage.

Greenfield and Wallace (1950) described the early changes of villonodular synovitis. Inflammatory components, including small lymphocytes, occasional plasma cells, and macrophages were found. Foreign body giant cells were rare or entirely absent. Large histiocytes or mononuclear cells, whose cytoplasm was vacuolated and foamy, were interspersed in the network.

Shafer and Larmon (1951) reported on seven cases. The descriptions were nearly identical to those of Jaffe et al. (1941). They agreed that the lesion was inflammatory in nature but reported that no specific cause could be determined.

Young and Hudecek (1954), in an effort to demonstrate that trauma was a significant factor in the etiology of villonodular synovitis, injected the stifle joints of dogs with uncitrated, autogenous blood at least once a week for a year. They concluded that "the presence of blood in joints is essential for the production of pigmented villonodular synovitis."

Breimer and Freiberger (1958), reported on the bone lesions associated with villonodular synovitis. These included cyst formation in the subchondral bone and erosion of articular cartilage and cortical bone. They expressed the opinion that the osseous lesions were initially the result of pressure from the soft tissue mass. This pressure caused erosion of the articular cartilage and of the cortical bone; the cystic areas within the bone were produced by expansion of the mass within the softer cancellous bone.

In the same year, Jaffe (1958) again described the clinical and pathologic appearance of the lesion. He maintained the view that intraarticular hemorrhage is not the basic underlying cause. He stated that in the villous, matted, and hypervascular synovial membrane of the hemophilic joint, the cytologic features of pigmented villonodular synovitis such as foam cells and multinuclear giant cells among nodular or diffuse collections of polyhedral stromal cells, were not represented. He did concede, however, that functional trauma or hemorrhage could act as exacerbating factors.

Recently, Robbins (1962) gave a precise histologic description: "Histologically, the inflammation consists principally of proliferation of subsynovial connective tissue and intense mononuclear leukocytic infiltration, accompanied by extensive deposition of granular hemosiderin pigment within the villous processes. Foci of lipophages in the fibroblastic stroma, hemosiderin pigment and numerous multinucleate foreign body type or osteoclastic giant cells may occur in some of these forms producing what is called by some a giant cell tumor. However, these lesions are unquestionably inflammatory reparative granulomas, perhaps a response to old blood and chronic irritation, and do not therefore merit the designation neoplasm."

Robbins stated that the etiology of both the diffuse synovitis and the localized giant cell granuloma of tendon sheath origin probably rests in some obscure cause of a chronic inflammation, such as repeated episodes of hemorrhage. Such an origin would explain the abundant lipoid and blood pigment and the essentially reparative characteristic of these lesions.

Degenerative Osteoarthritis

Proliferation of the synovial villi is only part of the changes noted on postmortem examination of animals which are suspected to have had intraarticular hemorrhage. Of far more importance are the articular lesions which appear to be the

type occurring in degenerative osteoarthritis.

Swanton (1959) reported the histopathology of hemophilic arthropathy in dogs. These findings provided the link between the literature on synovial hyperplasia and that on degenerative arthritis as related to intraarticular hemorrhage.

The most frequent and constant manifestation of canine hemophilia was recurrent lameness appearing early in life and sometimes accompanied by obvious joint swelling. These dogs also showed enlargement of the synovial villi with mild to moderate plasma cell and lymphocytic infiltration, hemosiderin laden phagocytes, hyperplasia of superficial synovial cells and adherent fragments of hyalinized fibrin. These joint changes might progress to fibrosis of the joint capsule to the degree that joint mobility could be severely restricted.

She observed that in joints with evidence of previous hemorrhage, the articular portions of the bone often had changes which were typical of the type occurring in degenerative osteoarthritis. In the less severe cases, these alterations included a dull, granular, velvety and soft articular cartilage. In the more severe cases, the changes ranged from pitting to focal grey or reddish eroded areas or extensive coarse roughening of the surface. Lipping or osteophyte formation at the articular margins was noted as were irregular cartilaginous and bony proliferations adjacent to the margins of the articular cartilage.

Keefer and Myers (1933) made like observations on the similarity between hemophilic arthritis and degenerative osteoarthritis.

In the early literature, there was confusion as to the classification of equine lamenesses. Degenerative osteoarthritis, as it is known today, was placed under the general designation of lameness.

Vivien and Augustin (1904) theorized that spavin was the same as osteoarthritis and was characterized by the growth of typical osteophytes at the articular margins.

Williams et al. (1905) included arthritis deformans, arthritis sicca, spavin, navicular disease, ringbone, sidebone, sesamoiditis, spinitis, gonitis, carpitis, humero-radial arthritis, scapulo-humeral arthritis, in the "spavin group of lamenesses." This was done on the basis of their feeling that this group of maladies was similar, if not identical, in cause and pathologic changes and was fundamentally a "constitutional" rather than a local problem. They felt that such conditions as strain, concussion, faulty shoeing, compression from tendons, labor or confinement, climate, heredity, rheumatism, contagious pneumonia, rachitis, osteoporosis and "osteomalacie" were immediate or precipitating causes of the "spavin group of lamenesses." Because of a generally debilitated skeletal system, these conditions were able to produce the lesions of the "spavin group of lamenesses."

However, spavin apparently differs from osteoarthritis in that the former is not primarily a degenerative disease of articular cartilage (Sokoloff, 1960).

Hare (1927) gave an account of equine chronic arthritis. His findings were essentially similar to those later reported for degenerative osteoarthritis. However, he believed that the initial lesions originated in the subchondral bone. He also suggested that the lesions were inflammatory in nature.

The lesions in the articular cartilage began over circumscribed, congested areas in the bone. Microscopically, he described a ragged appearance of the articular cartilage resulting from cleavage of the matrix into a mass of delicate filiform cylinders of cartilage. These clefts passed inward between vertically or obliquely distributed columns of matrix and cartilage cells and in the more advanced cases, penetrated into the zone of provisional calcification. He noted that, in spite of the cleavage of the ground substance, the cartilage cells themselves appeared healthy. However, near the surface, the cells seemed to multiply and appeared in clones of four to eight cells. The final stage of degenerative arthritis was ankylosis. The opposing eroded surfaces were found adherent through vascular fibrous tissue. Bony ankylosis was of minor importance.

In the synovial membrane, the initial change was hyperemia. The joint capsule became swollen and infiltrated with

yellowish-brown serous effusion. Areas of necrosis developed which, in a later stage, became surrounded by a peripheral zone of granulation tissue. There was no evidence for the presence of bacteria. Next, proliferation of the softer structures of the capsule occurred. The villi became enlarged, branched and increased in number. Granulation tissue in the form of fibroblasts replaced the necrotic foci and spread diffusely throughout the tissue. Phagocytic cells and an occasional giant cell were found. As the lesion developed, fibrous whorls appeared around blood vessels. At the marginal area between the synovial membrane and the articular cartilage, the initial changes were an increased prominence of the blood vessels with focal necrosis. From this, vascular connective tissue proliferated over the intact healthy articular cartilage, causing a "pannus" formation. In the advanced cases, eburnation of the bone was reported.

Callender and Kelser (1938) published their work in which they described and compared the lesions of degenerative osteoarthritis in the equine species with those in man. Longitudinal depressions in the articular cartilage which they referred to as "grooving", was one of the first changes seen. This was an infrequent finding in horses and rare in man. "Splits and clefts" occurred and extended from the "grooves" into the cartilage and even into the cortical bone. However, subchondral bone lesions were found only when prior cartilaginous

changes had reached the bony cortex. A somewhat more common finding was an elevation of the tangential zone under which was a vacuole which they referred to as "blister formation." According to these writers, when the "blisters ruptured," fringes were formed. The next stage in the progression of degenerative osteoarthritis was necrosis and ulceration. This was characterized by necrosis and loss of substance of the surface of the ulcer which was the most common finding in the equine species. In the horse, the bases of the deep ulcers were sometimes covered by a layer of dense fibrous tissue though masses of fibrillated hyaline cartilage may have remained projecting from the underlying bone. However, the bone itself was never bare. Hare (1927) differed on the latter point. Ulceration shifted the bearing surface which resulted in malocclusion of the surfaces. Hypertrophic changes, which appeared to be compensatory in character, then took place. These first appeared as a moderate degree of hypertrophy of the remaining cartilage at the margins of the bearing surface. Next, new bone formed which originated from the calcified matrix of the cartilage, extended into it and eventually became continuous with the old bone. These changes formed the lipping or osteophytes of degenerative osteoarthritis from which "joint mice" may originate. Callender and Kelser (1938) noted that these hypertrophic changes were not numerous or severe in horses because they were euthanized before advanced

changes could take place.

In man the lesions of the synovial membrane were usually confined to advanced hypertrophy. When present, the synovial fluid may have been of "dark color" and of gelatinous consistency. In horses, however, there was usually accompanying evidence of trauma complicating the disease process. The authors stated that the process originated in the peripheral one half to one third of the cartilage which receives its nutrition from the synovial fluid. It seemed logical to them that a change in character of the synovial fluid, rather than in the subchondral bone, was the primary degenerative process. They stated that the periosteal bone formation which occurred in spavin was not a part of the condition and that spavin was not a disease of the joint cartilage. This view was supported by Sokoloff (1960).

Important clinically was their observation that in both man and the horse, the extent and severity of the lesion did not always correspond to the clinical findings. These observations were later supported by others (Jenny, 1962; Sippel, 1942a). Callender and Kelser (1938) observed that in inflammatory conditions of the joint, pain and tenderness were present early in the course of the disease, often at the same time that pathologic changes first occurred. In degenerative osteoarthritis, however, clinical signs did not usually occur until after the process was advanced and secondary hypertrophic changes had taken place.

Etiologic Factors of Degenerative Osteoarthritis

Miscellaneous factors

A multitude of factors, singly or in combination, have been proposed as predisposing or etiologic factors of degenerative osteoarthritis. However, many of these factors were discussed during the confusion that existed on the relationship of the lesions of degenerative osteoarthritis to other "lamenesses."

Way and Hoffman (1935) stated that all the chronic lamenesses of horses were caused by bacterial infection; and they claimed excellent results using autogenous vaccine therapy.

Mitchell offered several explanations as to etiologic factors. He supported Way and Hoffman's infectious agent theory (Mitchell, 1931). He suggested that such conditions as "roaring, whistling, ringbone, sidebone, spavin, navicular disease, shivering, jinked back, and stringhalt" were the result of osteoarthritis of the spine, the exostoses causing nerve damage (Mitchell, 1930a). However, he later observed cases of "shivering" with no exostoses of the vertebrae (Mitchell, 1930b and 1937). He also included these conditions along with osteoarthritis under the term "rheumatic disease" (Mitchell, 1935 and 1937). He suggested that they were connected with diffuse peripheral neuritis, and described what he thought were the nerve lesions (Mitchell, 1933 and 1937). Sippel could not support this idea at first (Sippel,

1942a) and he later identified the "lesions" as normal Pacinian corpuscles (Sippel, 1942b).

Parker and Keefer (1935) compared the lesions of rheumatoid arthritis with those of osteoarthritis in man and found that no comparison existed in that rheumatoid arthritis was definitely inflammatory in nature whereas osteoarthritis was degenerative.

Callender and Kelser's paper (1938) suggested that "metabolic or rather chemical or physical-chemical changes in the cartilage render it more susceptible to damage by normal wear and tear."

Sippel (1942a) gave an excellent gross and microscopic description of equine degenerative osteoarthritis. He discussed the etiology according to the following types: subchondral, intraarticular, systemic, and neurogenic.

The subchondral type was based on Hare's (1927) theory that the site of origin of the lesions of "rheumatic arthritis" was in the subchondral bone. Here, he refers to Phemister (1940) who called attention to the production of cartilage defects by interruption of the circulation to portions of the articular cartilage.

Under the "intraarticular" heading, inflamed synovial linings and blood-stained synovia and cartilage were present in the joints of several horses that had died of acute or subacute diseases. Sippel considered vitamin or mineral

deficiency or imbalance as possible factors and stated: "we are of the opinion that the role of vitamin A, (alone or in combination with other vitamins), in equine degenerative arthritis warrants further investigation."

Trauma and "abnormal stresses" have been mentioned as etiologic factors by several investigators (Bennett, 1957; Sokoloff, 1960); especially by Mackay-Smith (1962).

Nutritional factors

Numerous workers have discussed the relationship of nutritional imbalances and deficiencies to equine lamenesses (Bardwell, 1961; Campbell, 1934; Crawford, 1939; Frost, 1934; Greenlee, 1939; Kelser and Callender, 1938; Kintner and Holt, 1932; Kintner, 1940; Mitchell, 1930b; Sippel, 1942a; Sokoloff, 1962; Trum, 1959). Many of these writers have either directly or indirectly associated joint lesions with osteomalacia. Kelser and Callender (1938) maintained that osteomalacia *per se* was not involved in the pathogenesis of degenerative osteoarthritis and pointed out that in the cases which they studied and called degenerative osteoarthritis, the bone was of normal hardness and the horses lacked other systemic signs of osteomalacia. They stated that irrespective of the precise cause of degenerative arthritis, they found no evidence that it was osteomalacia. However, they did not exclude the possibility that dietary deficiency or imbalance might be involved.

Trum (1959) clarified the relationship between osteomalacia and degenerative osteoarthritis by saying: "Facetiously, I consider that the concept of comparative pathology reached its zenith in the study of equine osteoarthritis. Kelser and Callender found that the pathology of equine osteoarthritis was closer to human degenerative diseases than to osteoarthritis of Equididae due to nutrition."

However, Sokoloff (1962) warned against emphatically excluding diet, as it affects the skeletal system, from the list of possible causes of degenerative osteoarthritis.

Age remodeling

The incidence of degenerative osteoarthritis increases with advancing age (Johnson, 1959 and 1962; Keefer et al., 1934; Moffet et al., 1962; Parker et al., 1934). Johnson (1959 and 1962) and Moffet et al. (1962) showed the relationship between osteoarthritis and the joint remodeling of growth and of age. Johnson (1962) stated that degenerative osteoarthritis resulted from decompensated or unbalanced remodeling. He listed ten factors which contribute to unbalanced remodeling leading to degenerative osteoarthritis. He also stated (1959) that "all the proliferative, secretory, shedding, and adaptive mechanisms of remodeling are active in osteoarthritis, at local sites of exaggeration." He classified osteoarthritis into "primary (i.e., without any special triggering mechanism) or secondary (i.e., following some

other joint damage)." Thus the type osteoarthritis of interest in the present study is secondary osteoarthritis which Johnson felt "results from altering any of the factors involved in cartilage growth, remodeling, and turnover." He gave a list of such factors, one of which is "the retention of organic products of the RBC membrane (particularly the polysaccharide and possibly the phosphatide moieties)." These, he maintained, "are more important than hemoglobin, hemosiderin, or iron in initiating the degenerative joint changes of hemophilia because of their action in altering synovial fluid chemistry and viscosity, and in modifying osmotic relationships for exchange between cartilage and synovial fluid."

Throughout this review, the similarity of degenerative osteoarthritis in the equine and in man has been stressed, as has its degenerative nature. However, Sokoloff (1962) stated, "Anatomic findings in the joints of horses with osteoarthritis reveals that hypertrophic villous synovitis is common. This is a feature of rheumatoid and infectious rather than traumatic or degenerative osteoarthritis in man. While one may conceive of a number of explanations for the disparity in the findings in the two species, the differences should be recognized in making any inferences on the etiology and genesis of this condition."

The literature reviewed covers other types of equine lamenesses. This has been done in the hope that it will, in some

way, clarify the position of degenerative osteoarthritis in relation to the other types of lamenesses.

This review also includes available representative literature concerning the presence of blood in the diarthrodial joint and has attempted to correlate it with the literature on degenerative osteoarthritis. In so doing, it has attempted to demonstrate the possible relationship between hemarthrosis and degenerative osteoarthritis.

METHODS OF PROCEDURE

The Experimental Animals

Eight grade Shetland ponies ranging in age from 12 to 24 months and weighing from 200 to 350 pounds were purchased from farmers and horsemen in the Ames, Iowa, area. No previous history was available for these animals. They were purchased as clinically sound and having no evidence of abnormal articulations.

Immediately after purchase, blood and fecal samples were collected and studied. The blood study included a total erythrocyte count, total leukocyte count, leukocyte differential count, and hemoglobin and microhematocrit tests. Four of the animals were found to be anemic and were placed on a two-week course of oral iron therapy. Two of them (Department of Veterinary Pathology numbers 64-R-171[1] and 64-R-172) did not respond well so the treatment was continued for an additional two weeks. At the beginning of the experiment, animal 171 had not responded and animal 172 was responding slowly but was still slightly anemic.

All of the animals were found to have Strongylus spp.

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[1]A letter (e.g. D or R) was assigned to each animal during the experiment. However, for purposes of convenience and because a permanent record is available, the Department of Veterinary Pathology number will be used throughout this paper. The abbreviated form will be used (e.g. animal 171 instead of No. 64-R-171). Table 1 gives the correlation between the experimental and the departmental number.

Table 1. Injection schedule

Experimental no.	Path. Dept. no.	Time postinjection	Material and amount of material injected			
			Right radiocarpal	Left radiocarpal	Left femorotibial	Right femorotibial
L	64-R-128	15 d[a]	15 ml saline	15 ml blood	75 ml blood	5 ml blood
D	64-R-171	1 m[b]	13 ml serum	" " "	" " "	" " "
R	64-R-172	1 m	15 ml saline	" " "	" " "	" " "
DL	64-R-173	2 m	15 ml saline	" " "	" " "	" " "
MR	64-R-174	2 m	13 ml serum	" " "	" " "	" " "
V	64-R-175	3 m	13 ml serum	" " "	" " "	" " "
M	64-R-176	3 m	15 ml saline	" " "	" " "	" " "
VM	64-R-438	Control	Control	Control	Control	Control

[a] d = days.

[b] m = month(s).

infection and were treated with an anthelmintic twice at two week intervals.

The animals were housed in individual stalls bedded with straw. Good quality alfalfa hay and water was provided ad libitum. A small amount of grain was given daily. During the day, when the weather was not too severe, the ponies were taken to pasture for a period of four to eight hours.

The joints to be injected were examined radiographically using two views (anterior-posterior and lateral-medial) per joint. All joints were found free of radiographic evidence of joint involvement.

When the animals were considered clinically healthy, the experiment was begun. In the case of animals 171 and 172, it was felt that it was not advantageous to wait for further elevation of erythrocytic numbers.

Experimental Design

The experiment was designed to study the sequential effects of autogenous blood in joints, over a three month period. Seven animals were injected. Of these, one was killed at 15 days, two at one month, two at two months, and two at three months postinjection. The remaining animal served as an uninjected control.

In each experimental animal, the left radio-carpal joint was filled to its maximum (approximately 15 ml.), under pressure, with autogenous blood. The same was true with the left

femoro-tibial joint (approximately 75 ml.). The right femoro-tibial joint of each animal received five ml. of blood. The right radio-carpal joint was injected with either 15 ml. of sterile 0.85% NaCl (saline) or autogenous serum (approximately 13 ml.) according to Table 1.

The day prior to injection, blood was collected from animals 171, 174, and 175. This blood was allowed to clot and from it the serum was collected. The serum was then centrifuged at 2500 rpm for 20 minutes and was recollected. In this entire procedure, sterilized glassware was used. The serum yield was 13 ml. in each case. An antibiotic mixture of penicillin and streptomycin (1000 units of penicillin and 500 micrograms of streptomycin per ml. of antibiotic mixture) was added to the serum to give a final dilution of one part of antibiotic mixture to nine parts of serum. This was then incubated at 37 C for 30 minutes and at room temperature for two hours and refrigerated until used.

Injection Procedure

The entire ventral aspect of the cervical region and a generous area around both carpal and both femoro-tibial joints were clipped with a No. 40 Oster[1] blade. These areas were scrubbed twice with soap and water. The animal was next

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[1]John Oster Manufacturing Co., Milwaukee 17, Wisconsin.

placed under general anesthesia, using Equithesin[1], and positioned in dorsal recumbency. Ropes were fastened to the fetlock and pastern area of each hind leg which was then held in about seven-eighths flexion. The forelegs fell passively into complete flexion. The neck and joints were again thoroughly scrubbed. Two applications of 40% ethyl alcohol and two applications of merthiolate were used. The bacteriostatic material was allowed to dry between each application. Next, the operators "scrubbed" and put on rubber gloves.

The sites of injections were as follows: for the radio-carpal joints, just lateral to the tendon of the extensor carpi radialis and at the point of deepest depression between the radius and the radial-carpal bone; for the femoro-tibial joints, just anterior to the lateral patellar ligament and just above the proximal end of the tibia.

The proposed site of injection was next determined and a one cm. skin incision was made. This was followed by another application of merthiolate.

A one inch, 18 gauge hypodermic needle was inserted through the skin incision and into the right radio-carpal joint. The hub of the needle was covered with three sterile gauze pads while serum or saline was being aspirated into the syringe. The syringe was attached to the hub of the needle

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[1]Jensen-Salsbery Laboratories, Kansas City, Missouri.

and the solution was injected. The syringe, with needle still attached, was withdrawn.

Next, a one inch, 18 gauge needle was placed into the left radio-carpal joint and two inch, 18 gauge needles were inserted through the skin incision into the lateral sac of each femoro-tibial joint. After placement, the hub of each needle was covered with three sterile gauze pads.

A two inch, 16 gauge needle was inserted into the left jugular vein. Sterile gauze pads were placed under and over the hub.

Using two operators, blood was withdrawn from the jugular vein into a sterile 20 ml. syringe and injected into the various joints in amounts previously stated. When the left femoro-tibial joint approached maximum capacity, the pressure from within the joint cavity forced the blood through the needle after the syringe was detached. This was prevented by placing a sterile gauze pad over the hub while the second operator was aspirating more blood from the jugular vein. Immediately after a joint injection was completed, the needle was withdrawn and the wound was swabbed with merthiolate. The animals were given 1500 units of tetanus antitoxin and then moved to their stalls.

Postinjection Period

After about four days, during which time the animals were continually in their stalls, they were taken to pasture

during the day for four to six hours, weather permitting.

The animals were observed daily for clinical signs of lameness. In addition, the joints were palpated for evidence of distension and elevation of local skin temperature.

On the day prior to necropsy, blood for a hemogram was collected from each animal. A second radiographic examination was conducted.

Necropsy Procedure

The animals were taken to the necropsy laboratory. Both carpi were wrapped with a thick layer of towels. The animals were then killed by electrocution.

All the joints were opened and sterile-swab samples were collected for bacteriologic study.

All the injected joints and the right tibio-tarsal joint (individual uninjected control), were completely opened, blotted clean with a moist towel, photographed; then samples of synovial membrane and articular cartilage with underlying bone were collected and placed in 10% buffered formalin.

Histologic Procedure

The cartilage samples had been cut to include a generous portion of the underlying bone. They were cut at a thickness of approximately five mm. Both bone and synovial membrane samples were then placed in 10% buffered formalin and fixed for from five to seven days. At this time, the specimens

were trimmed and the bone samples were put into nitric acid decalcifying solution under a negative pressure of approximately 18 mm. of mercury[1]. At this time the synovial membrane samples were embedded using a procedure of the Armed Forces Institute of Pathology (1960).

The decalcifying solution was changed every three to four days. After about one week, the bone was removed from the decalcifying solution and each specimen was cut in half with a razor blade. The specimens were then replaced in the decalcifying solution, and allowed to stand about one week more. After decalcification, the cartilage-bone samples were then embedded using the same procedure as was used for the synovial membrane.

After embedding, all samples were cut at a thickness of approximately six microns. The synovial membrane sections were stained with Harris' hematoxylin and eosin; and with Turnbull's blue method (Armed Forces Institute of Pathology, 1960) for the presence of hemosiderin. Bone and cartilage sections were stained with Harris' hematoxylin and eosin; also with toluidine blue for metachromasia of cartilage.

The toluidine blue staining technique as outlined by Bélanger and Hartnett (1960) was used except the staining time

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[1]E. D. Roberts, Assistant Professor, Department of Veterinary Pathology, Iowa State University of Science and Technology, Ames, Iowa. A faster method for decalcifying bone. Private communication. 1964.

was five minutes instead of ten minutes. Tertiary butyl alcohol was used in dehydration in place of ethyl alcohol. Tertiary butyl alcohol does not destain toluidine blue as does ethyl alcohol.

When prominent changes of cartilage disruption were seen, it was felt necessary to rule out the possibility of artifacts incurred during sectioning. This was done by cutting other blocks of tissue as well as by re-embedding and re-sectioning the original block. If the changes were still present in the same location, they were reported.

Bacteriologic Examination

Two blood agar plates were streaked with the swab from each joint. One was incubated at 37 C under aerobic conditions and the other at 37 C under anaerobic conditions. Growth was looked for at 24 and 48 hours, Gram's stains were made, and differential media were inoculated from any of the colonies. No cultures were found to include pathogenic microorganisms.

RESULTS

Clinical Observations

The animals were very stiff and had difficulty rising to their feet on the day following injection. Pain was evidenced by very slow and careful walking in an effort to minimize movement of the joints. These signs had decreased in severity by the second day and on the fourth day rapidly disappeared following pasture exercise. During this time, the joints, with the exception of the right femoro-tibial, were very distended. Mild distension persisted for approximately one month post-injection. An elevated external skin temperature was detected over the injected joints during the first week. Lameness was directly related to the volume of material injected, inversely related to postinjection time and was not related to the type of material injected.

Gross and Microscopic Observations

Uninjected joints

Articular cartilage and synovial membrane of the radio-carpal, femoro-tibial and tibio-tarsal joints of one animal and the tibio-tarsal joints of seven animals, served as uninjected control tissues for this study. Twelve uninjected joints were examined.

Synovial membrane The synovial membrane was smooth and white to slightly yellow.

Microscopic examination revealed that in some areas, the luminal surface was villous; in other areas, it had a smooth appearance. The synovial cells were concentrated at the luminal surface. However, there was usually no distinct synovial cell layer at the surface. The nuclei of the synovial cells were round to oval and densely basophilic; the cytoplasm was indistinct (Figures 1 and 2). There was a transition of the loose areolar or adipose tissue of the villar and sub-villar stroma to the dense fibrous tissue of the joint capsule.

Articular cartilage The cartilage was smooth, firm in texture, and translucent in appearance. The color was white with a faint bluish tinge (Figure 3).

Histologically, the articular cartilage may be divided into three zones (Figures 4 and 5). The tangential (surface) zone contained small spindle-shaped chondrocytes which were oriented parallel to the surface. They had prominent, densely basophilic nuclei and indistinct, eosinophilic cytoplasm. The tangential matrix of the weight bearing portion was eosinophilic and homogeneous. However, near the marginal area (junction of articular cartilage and synovial membrane) of the joint, there was a gradual transformation of the hyaline matrix to fibrous tissue.

In the germinal (middle) zone, large round chondrocytes were arranged in columns which were more or less perpendicular to the surface. Clones of two to three of these cells were

Figure 1. Synovial villi of a radio-carpal joint of the control animal. Note the spindle-shaped synovial cells with very dense nuclei. Hematoxylin and eosin stain. X 230.

(Animal #438. Left radio-carpal joint; uninjected control.)

Figure 2. Higher magnification of the surface of a synovial villus shown in Figure 1. Note the absence of a definite synovial cell layer. Hematoxylin and eosin stain. X 800.

(Animal #438. Left radio-carpal joint; uninjected control.)

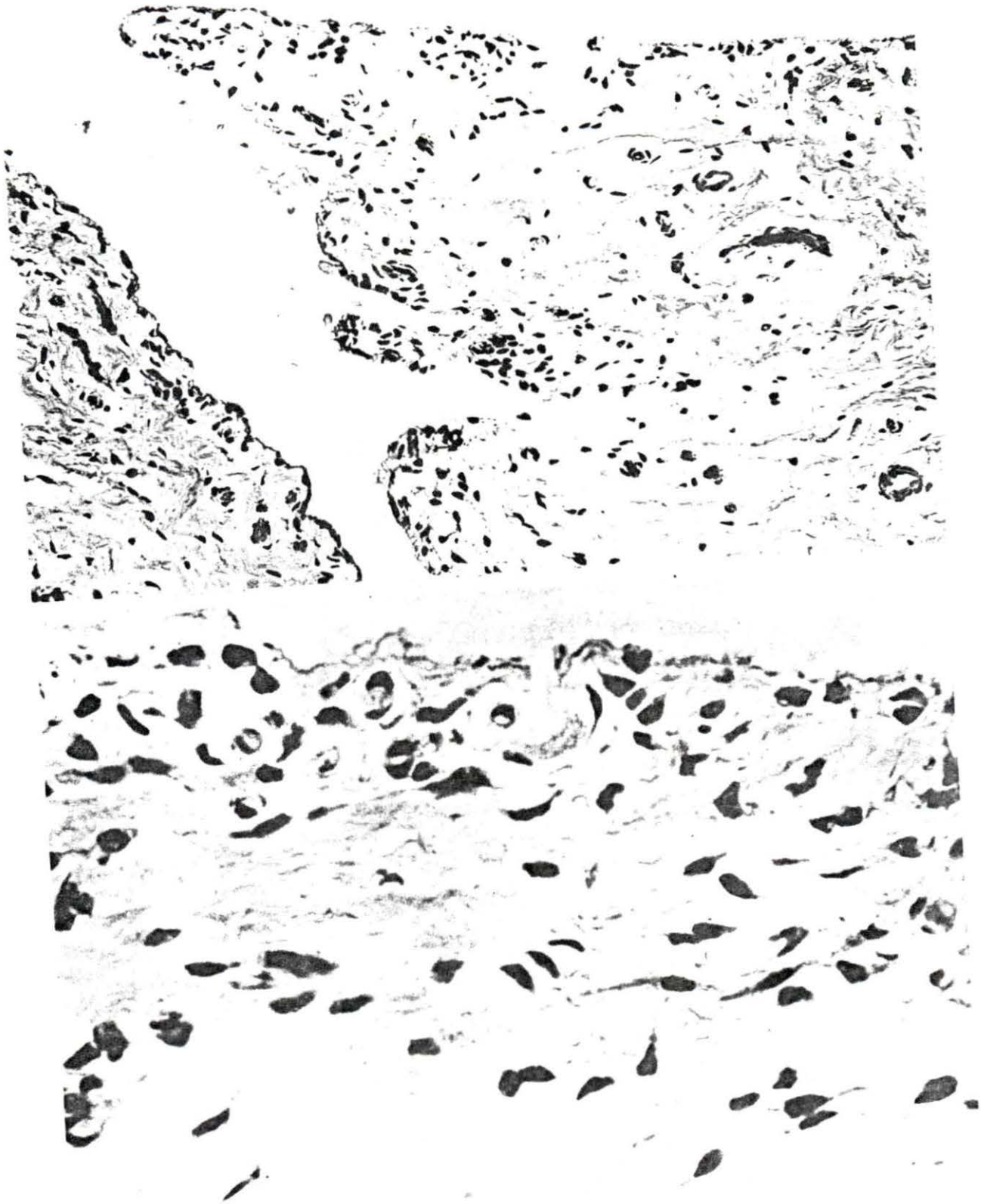


Figure 3. Articular cartilage of the control animal. The cartilage is white with a bluish tinge and has a smooth appearance. The synovial membrane is white to yellow and also has a smooth appearance.

(Animal #438. Distal end of femur; uninjected control.)

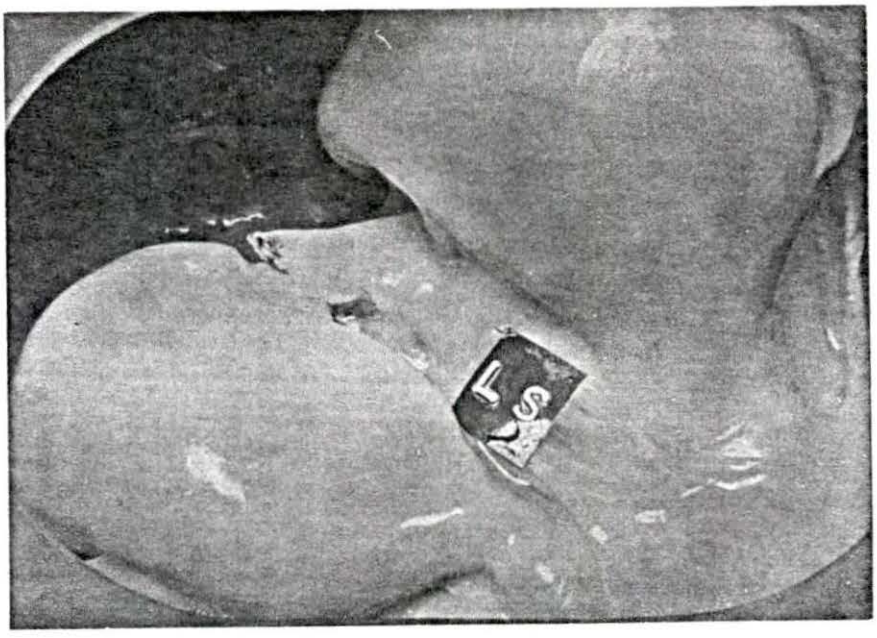
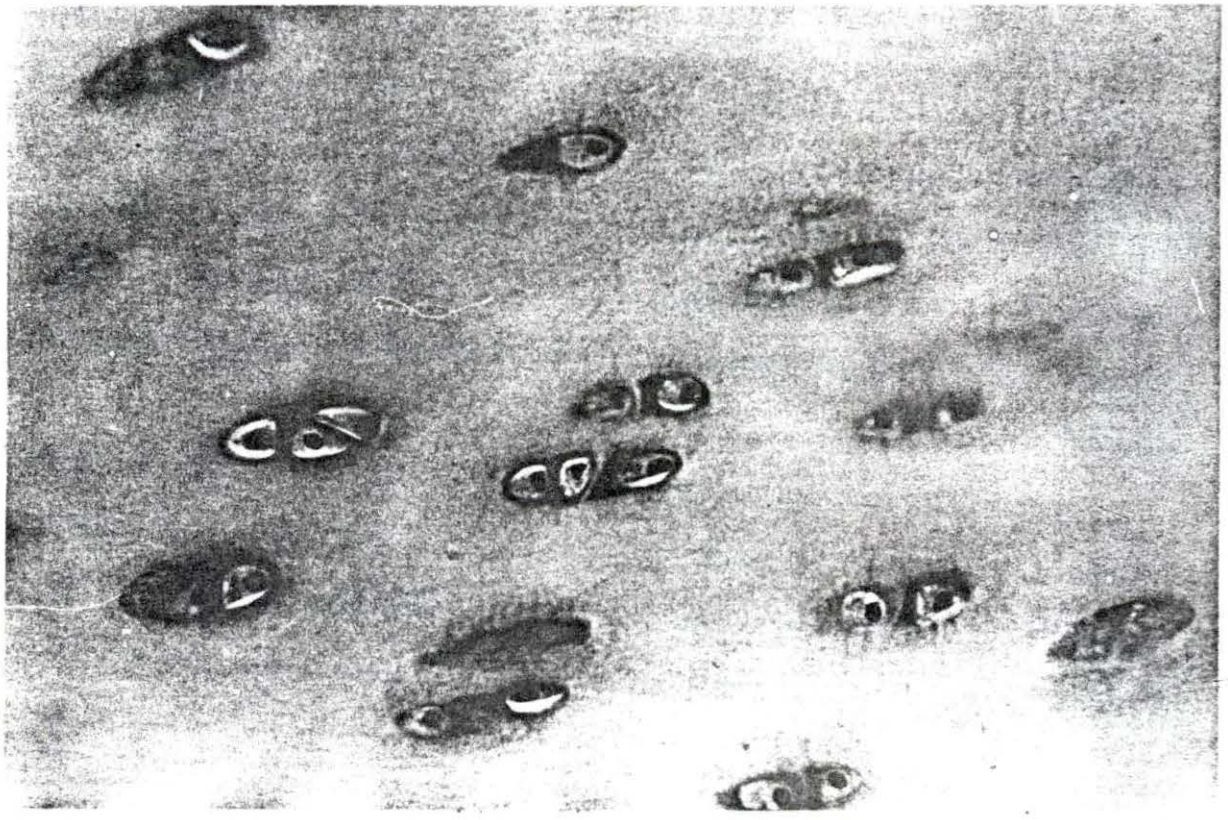


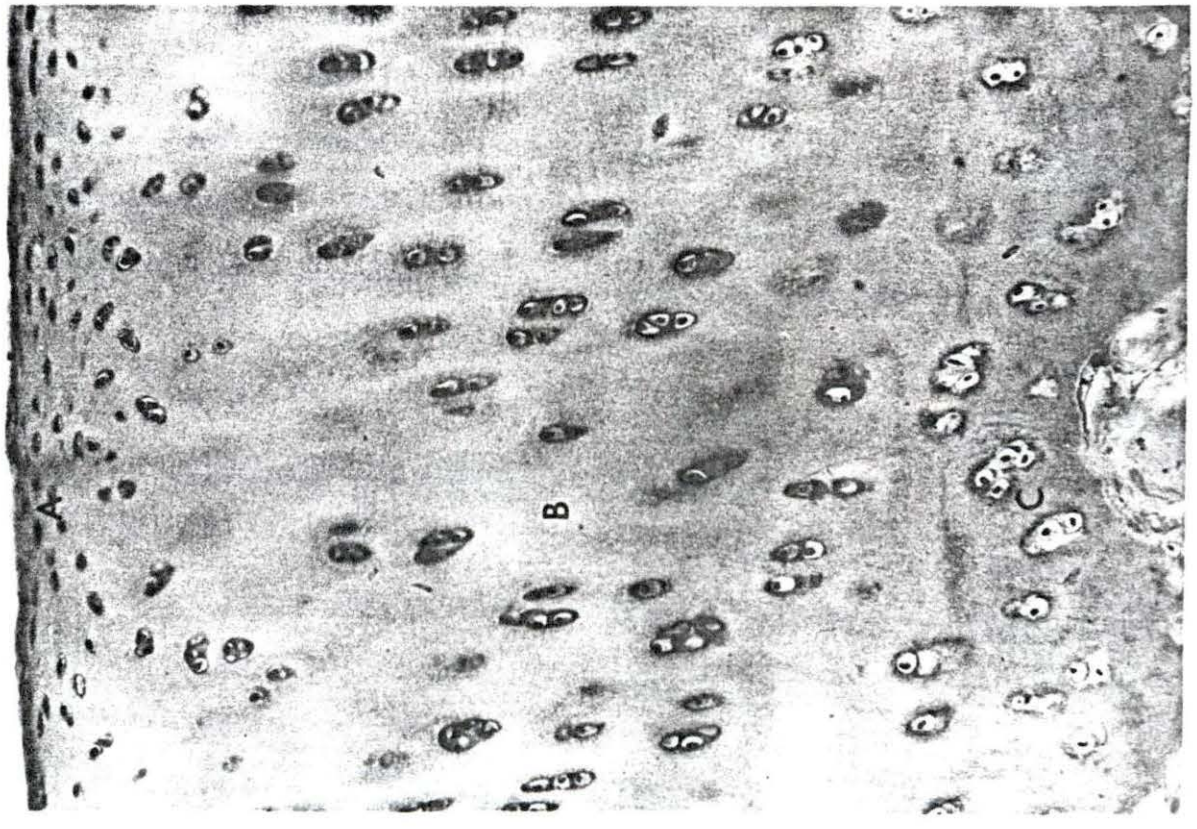
Figure 4. Left. Articular cartilage of control animal. The tangential zone (A), the germinal zone (B), and the zone of provisional calcification (C) are evident. Hematoxylin and eosin stain. X 315.

(Animal #438. Right radio-carpal joint; uninjected control.)

Figure 5. Right. Higher magnification of the germinal zone shown in Figure 4. Note the dark nuclei and prominent cytoplasm. Hematoxylin and eosin stain. X 785.

(Animal #438. Right radio-carpal joint; uninjected control.)




A
B

often seen. The staining reaction of the nuclei varied from eosinophilic to basophilic. The cytoplasm was prominent and homogeneous except for basophilic granules which were noted in two of the eight tibio-tarsal joints. The eosinophilic homogeneous matrix was modified in its staining properties so that the chondrocytes were surrounded by a basophilic cast (Figure 4).

Pyknosis and karyorrhexis of the nuclei of the chondrocytes of the (deep) zone of provisional calcification often resulted in debris-filled lacunae. The matrix of this zone was more basophilic than that of the outer two zones.

Injected joints

The right radio-carpal joint of each animal received either 15 ml. of sterile saline or 13 ml. of autogenous serum. The left radio-carpal joint received 15 ml. of autogenous whole blood; the left femoro-tibial received approximately 75 ml. of blood; and the right femoro-tibial joint received five ml. of blood.

Synovial membrane

Right radio-carpal (serum or saline) No gross or microscopic alterations were observed in this joint whether it received serum or saline (Figure 6).

Left radio-carpal (15 ml. of blood) The synovial membrane at 15 days postinjection had a yellowish discoloration which was most prominent on the anterior surface of the

joint cavity. The nuclei of most of the synovial cells were enlarged and hypochromatic (Figure 7). A large amount of hemosiderin was present in macrophages and in synovial cells (Figure 8).

Alterations similar to those noted at 15 days postinjection were present at one and two months postinjection. In addition, perivascular infiltration by lymphocytes and plasma cells (Figures 9 and 10) was observed in the villar and sub-villar areas of the membrane of animal 174 at two months postinjection. These cells had not phagocytized hemosiderin (Figure 11).

The only lesion observed at three months postinjection was in the sub-villar tissue of animal 175. This consisted of a focus of large foamy macrophages which contained a light brown noniron pigment. A small amount of hemosiderin was detected in some macrophages by Turnbull's blue reaction.

Left femoro-tibial (75 ml. of blood) The changes noted in this joint were similar to those of the left radiocarpal joint with the following exceptions: (1) nuclear changes of the synovial cells were first noted one month postinjection; (2) perivascular cuffing was found at one month postinjection.

Right femoro-tibial (5 ml. of blood) Perivascular cuffing in the sub-villar area by lymphocytes and plasma cells was observed at 15 days postinjection. Hemosiderin was

Figure 6. Synovial villi with normal appearing synovial cells. Compare with Figures 1 and 7. Hematoxylin and eosin stain. X 300.

(Animal #171. Right radio-carpal joint; injected with 13 ml. of serum. One month postinjection.)

Figure 7. Synovial villus with enlarged, hypochromatic nuclei of the synovial cells. Compare with Figure 6. Hematoxylin and eosin stain. X 305.

(Animal #171. Left radio-carpal joint; injected with 15 ml. of blood. One month postinjection.)

Figure 8. Hemosiderin in macrophages (M) and synovial cells (S). Turnbull's blue stain. X 195.

(Animal #173. Left radio-carpal joint; injected with 15 ml. of blood. Two months postinjection.)

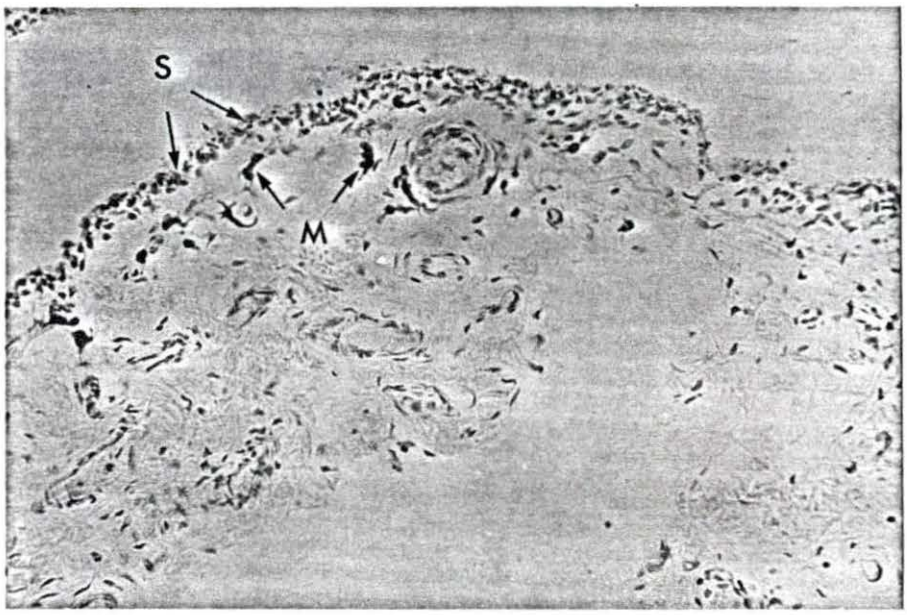

S
M

Figure 9. Perivascular cuffing by plasma cells and lymphocytes in the synovial membrane. Hematoxylin and eosin stain. X 77.

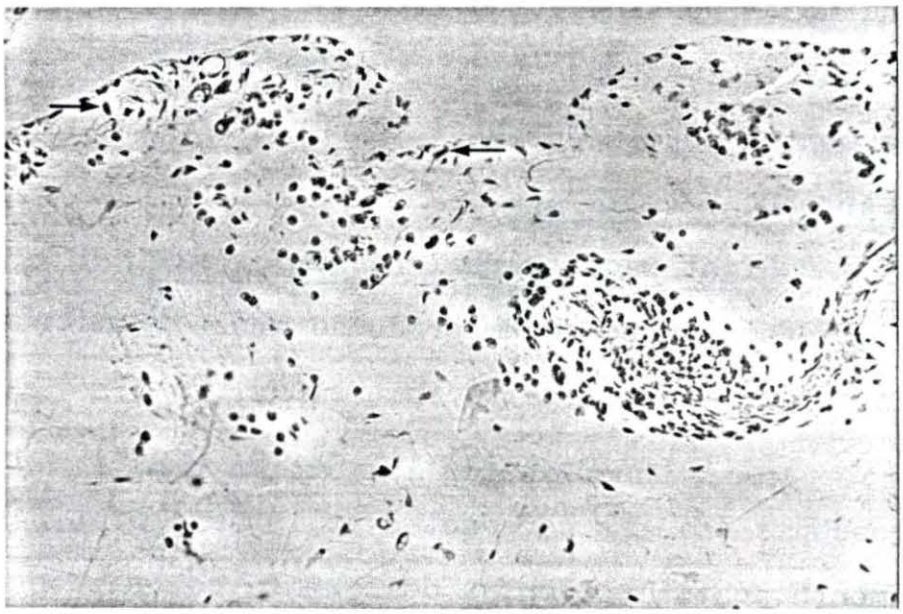
(Animal #172. Left femoro-tibial joint; injected with 75 ml. of blood. One month postinjection.)

Figure 10. Higher magnification of perivascular cuffing in synovial villus in Figure 9. Hematoxylin and eosin stain. X 330.

(Animal #172. Left femoro-tibial joint; injected with 75 ml. of blood. One month postinjection.)

Figure 11. Hemosiderin in synovial cells (arrows). Note that the inflammatory cells cuffing blood vessels do not contain the pigment. Turnbull's blue stain. X 120.

(Animal #172. Left femoro-tibial joint; injected with 75 ml. of blood. One month postinjection.)



present in macrophages.

A few nuclei of the synovial cells were enlarged and hypochromatic at one month postinjection. Hemosiderin was detectable only by Turnbull's blue reaction. Perivascular cuffing was noted in the sub-villar area of both joints.

The alterations noted at two months were similar to those at one month postinjection with the exception that no perivascular cuffing was observed. No alterations were noted at three months postinjection.

Articular cartilage

Right radio-carpal (serum or saline) Neither saline nor autogenous serum produced gross or microscopic lesions during the course of this study.

Left radio-carpal (15 ml. of blood) No gross or microscopic changes were noted at 15 days postinjection. At one month postinjection, a dull white coloration of the cartilage was observed. Microscopic alterations were confined to the upper germinal zone and lower tangential zone and consisted of chondrocytic degenerative changes of pyknosis and cytoplasmolysis. Surrounding a few of these cells, the matrix was granular and intensely eosinophilic (Figures 12 and 13) and with toluidine blue stain, was orthochromatic.

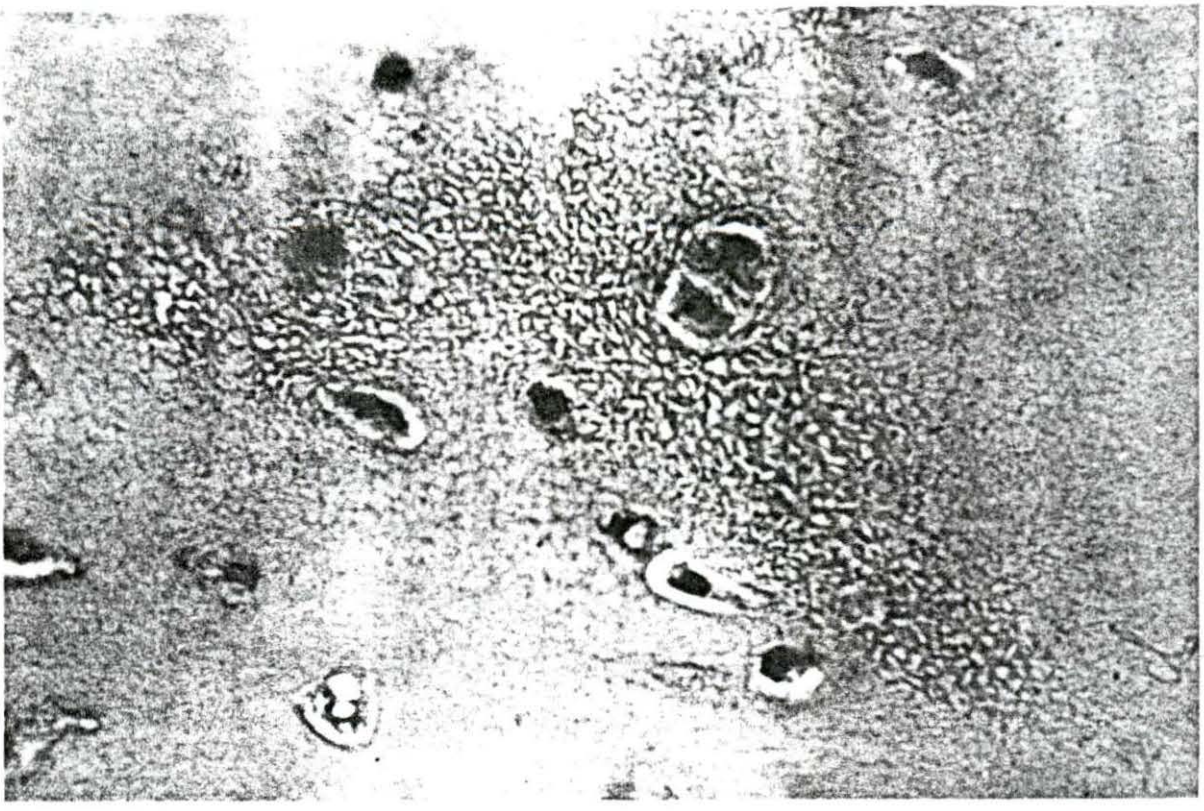
The lesions seen at one month were also present at two months postinjection. In addition, one or two small red foci (two to four mm.) near the anterior edge of the articular

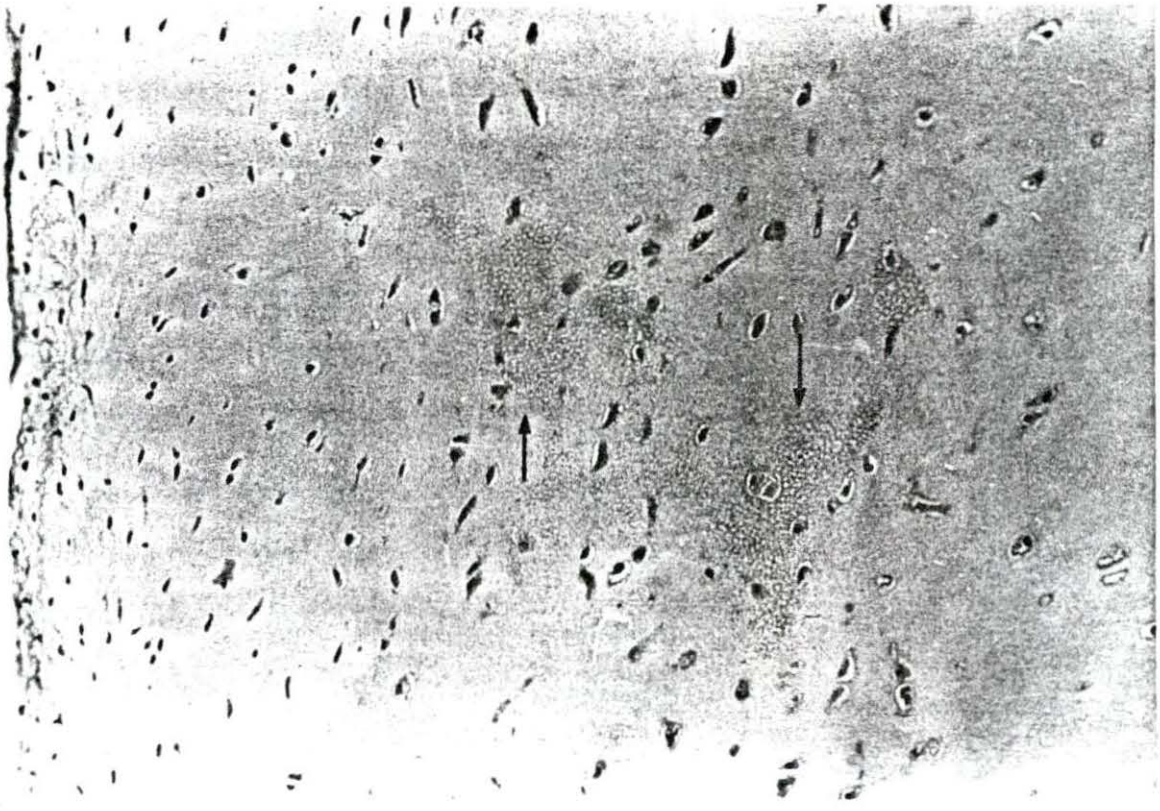
Figure 12. Left. Degenerative changes of irregularly oriented chondrocytes in the germinal zone of the articular cartilage. Note the granular matrix (which was intensely eosinophilic). Hematoxylin and eosin stain. X 255.

(Animal #171. Right femoro-tibial joint; injected with five ml. of blood. One month postinjection.)

Figure 13. Right. Higher magnification of the granular area in Figure 12. Hematoxylin and eosin stain. X 900.

(Animal #171. Right femoro-tibial joint; injected with five ml. of blood. One month postinjection.)





surface were seen on gross examination. A greater number of degenerated chondrocytes was present and they were more frequently associated with matrix changes. The articular cartilage of animal 174 had an area of marked fibrillation with loss of cellularity (Figure 14) which was associated with a loss of metachromasia.

At three months postinjection, the articular cartilage was soft. The anterior one half of the surface had a faint yellowish-red mottling while the posterior one half was dull white. The mottling was most pronounced in animal 175. Microscopically, erosion and fibrillation was present (Figure 15). Degenerative chondrocytes with associated matrix changes were more frequent than at previous samplings. At the margin of the joint in animal 175, focal metaplasia of the hyaline cartilage to fibrous tissue was observed. This fibrous tissue also infiltrated the cartilage-bone junction from the marginal area (Figure 16).

Left femoro-tibial (75 ml. of blood) There was a one square cm. area of slight yellowish-red discoloration on the medial aspect of the lateral condyle of the tibia at 15 days postinjection. Fissures, which extended through the tangential zone into the germinal zone, were observed near the margins of the articular surface. The edges of the fissures were smooth. The matrix surrounding them was less cellular, and when stained with toluidine blue, was orthochromatic.

The central one third of the cartilage of each condyle was faintly red and the remaining surface was dull white at one month postinjection. Chromatolysis, karyorrhexis and cytoplasmolysis were present in the deeper portion of the tangential zone and in the upper portion of the germinal zone and were often associated with the previously described matrix changes. In animal 171, basophilic stippling was noted in the matrix surrounding a few of these degenerated cells (Figure 17). Disruption of the perpendicular orientation of the chondrocytes was observed in some areas of the upper germinal zone. These lesions were in the weight bearing portion of the articular surface and were not associated with a loss of metachromasia.

The articular surface at two months postinjection was more eroded, softened and reddened than at one month postinjection. The chondrocytic degenerative changes were more numerous and more often associated with the surrounding matrix changes. At this time, the matrix changes were associated with a loss of metachromasia and an absence of basophilic stippling.

The gross and microscopic changes noted three months postinjection were similar to those of the two month group but were of a more advanced nature (Figures 18 and 19). Grossly observable fissuring was present by this time (Figure 18). In addition, more advanced microscopic lesions had occurred.

Figure 14. Upper left. Marked fibrillation and loss of cellularity at the articular surface. Hematoxylin and eosin stain. X 315.

(Animal #173. Left radio-carpal joint; injected with 15 ml. of blood. Two months postinjection.)

Figure 15. Upper right. Erosion and fibrillation of the articular surface. Note the granular matrix at the surface (arrows) (which was intensely eosinophilic). Hematoxylin and eosin stain. X 190.

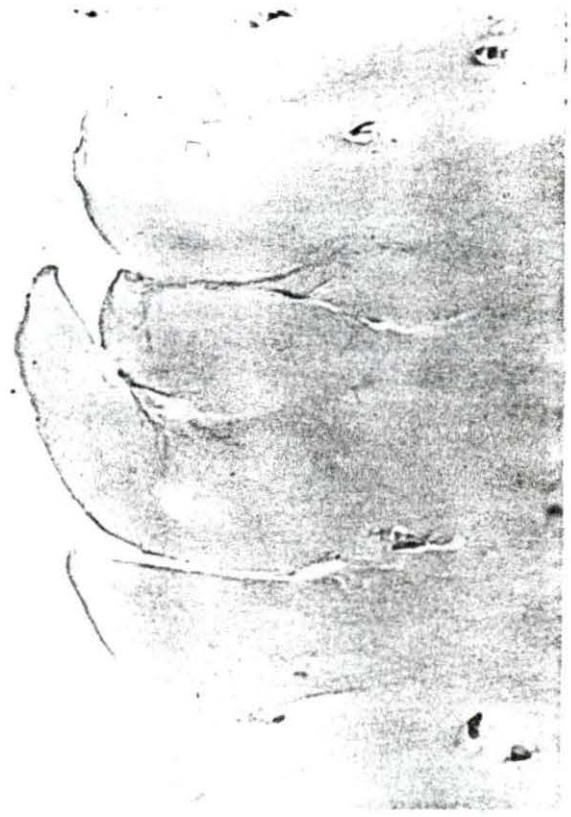
(Animal #173. Left radio-carpal joint; injected with 15 ml. of blood. Two months postinjection.)

Figure 16. Lower left. Focal metaplasia of hyalin cartilage to fibrous tissue near the marginal area of the articular surface (A), in the germinal zone (B), and at the cartilage-bone junction (C). Hematoxylin and eosin stain. X 185.

(Animal #175. Left radio-carpal joint; injected with 15 ml. of blood. Three months postinjection.)

Figure 17. Lower right. Degenerative chondrocytes with basophilic stippling (arrows) of the surrounding matrix in the germinal zone of the articular cartilage. Hematoxylin and eosin stain. X 545.

(Animal #172. Right femoro-tibial joint; injected with five ml. of blood. One month postinjection.)



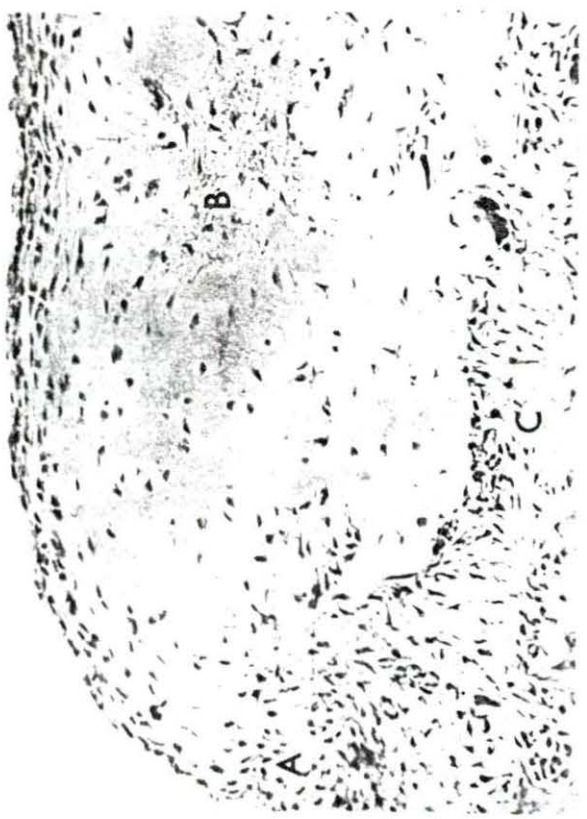
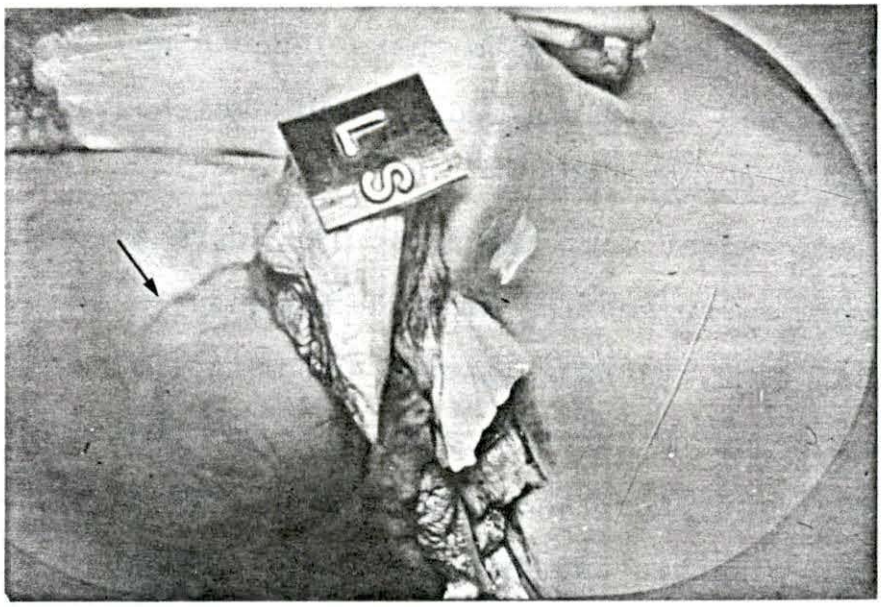

A
B
C

Figure 18. Articular cartilage with reddish-yellow mottling of the central one third of the articular surface. Note the fissure in the articular surface (arrow).

(Animal #176. Proximal end of tibia, left femorotibial joint; injected with 75 ml. of blood. Three months postinjection.)

Figure 19. Articular cartilage with reddish-yellow mottling of the cartilage on the medial half of each condyle.

(Animal #176. Distal end of femur, right femorotibial joint; injected with five ml. of blood. Three months postinjection.)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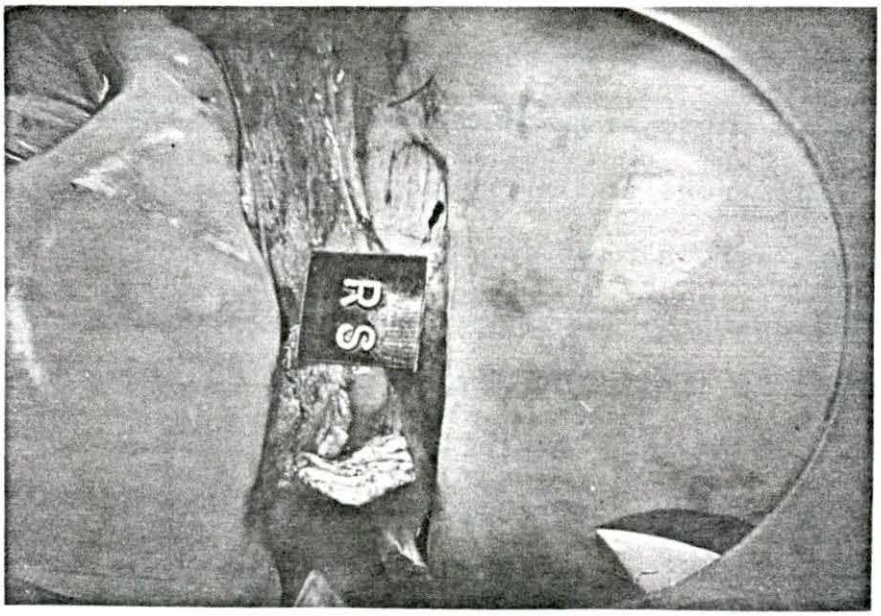

R
S

Erosions (Figures 20, 21 and 22) and fissures (Figure 23) of the articular surfaces of both the tibia and the femur were present. Associated with these lesions was a reduced number of cells per unit area and a matrix which stained orthochromatically (Figure 22).

A focal area of the articular surface was homogeneous and basophilic, suggesting mineralization. In the adjacent tangential zone, vascularization and metaplasia to fibrous tissue had occurred (Figures 24 and 25). In the germinal zone underlying this lesion, the chondrocytes were irregularly arranged and had pyknotic and cytoplasmolytic changes.

Right femoro-tibial (5 ml. of blood) No gross changes were observed 15 days postinjection. Microscopic examination revealed large focal areas of cytoplasmolysis of the chondrocytes in the upper germinal zone. The chondrocytes in these areas had lost their columnar orientation.

The gross and microscopic alterations one month postinjection were almost identical to those of the left femoro-tibial joint of the same postinjection time. The matrix changes in the right femoro-tibial joint were associated with a loss of metachromasia.

Gross and microscopic observations at two months postinjection were similar to those in the left femoro-tibial joint. Fissures which extended from the articular surface into the germinal zone were observed microscopically.

Figure 20. Marked erosion (arrow) of the articular surface as evidenced by a reduced thickness of the articular cartilage. Hematoxylin and eosin stain. X 72.

(Animal #175. Left femoro-tibial joint; injected with 75 ml. of blood. Three months postinjection.)

Figure 21. Higher magnification of the erosion shown in Figure 20. Note the degenerated chondrocytes in this area. Hematoxylin and eosin stain. X 310.

(Animal #175. Left femoro-tibial joint; injected with 75 ml. of blood. Three months postinjection.)

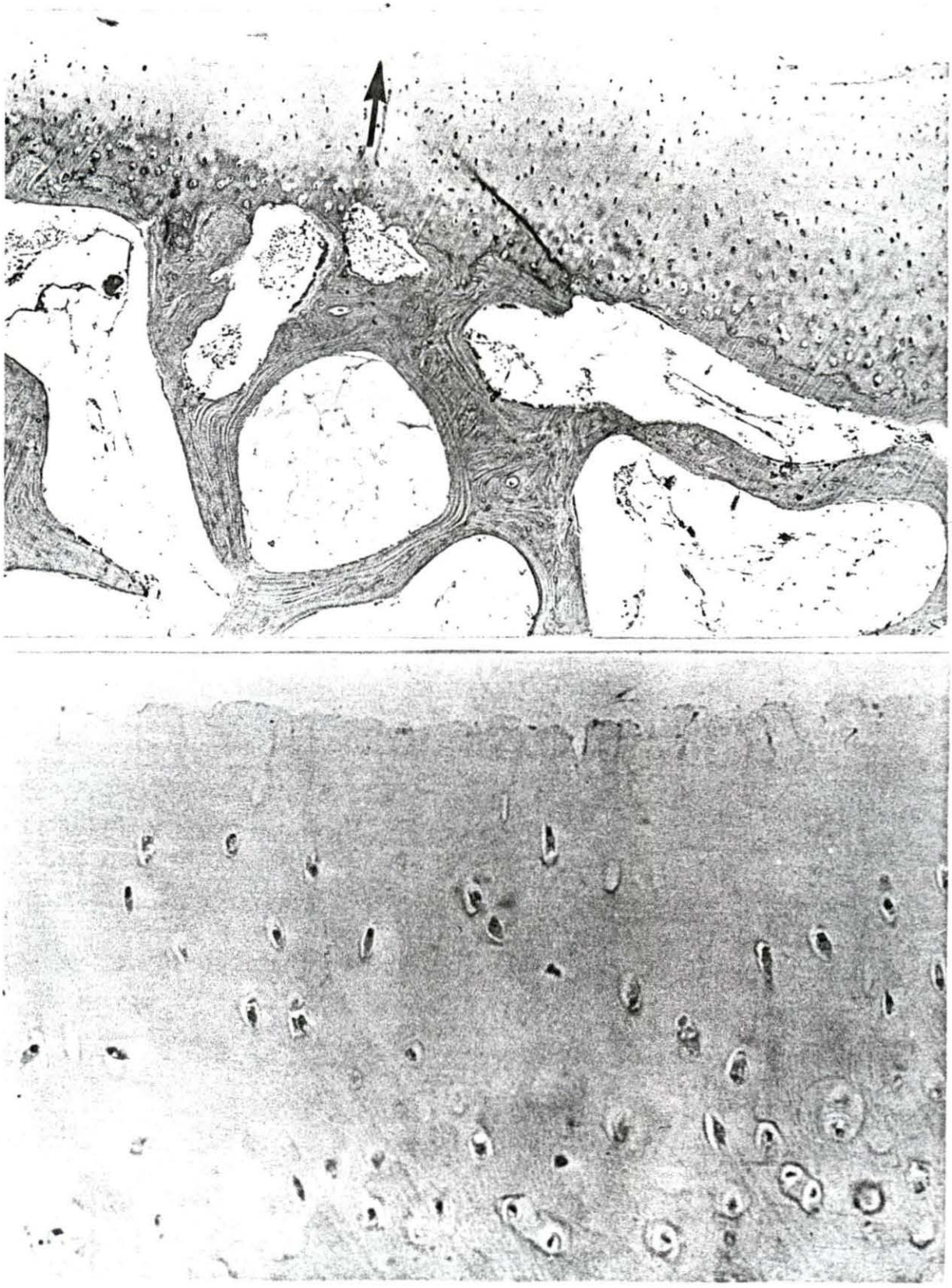


Figure 22. Loss of metachromasia at the surface of the eroded cartilage shown in Figure 21. Toluidine blue stain. X 195.

(Animal #175. Left femoro-tibial joint; injected with 75 ml. of blood. Three months postinjection.)

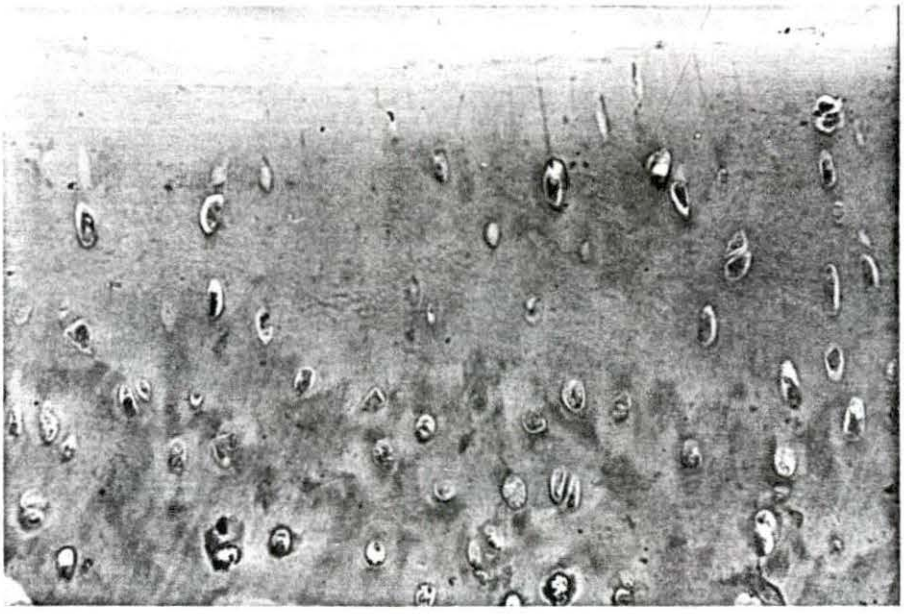
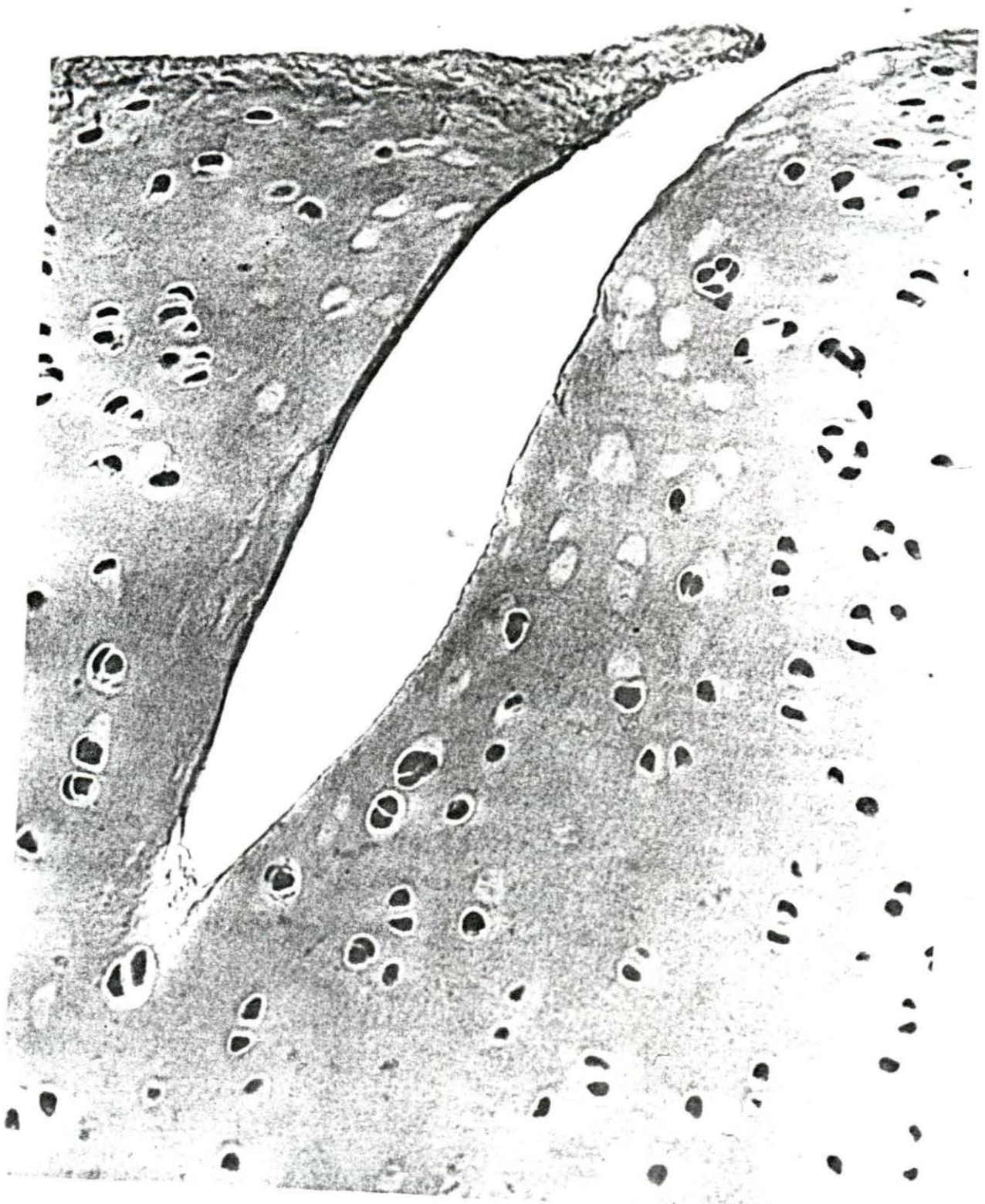


Figure 23. Fissure in the articular cartilage. Note the decreased cellularity and the vacant lacunae at the margins of the fissure. Hematoxylin and eosin stain. X 115.

(Animal #128. Left femoro-tibial joint; injected with 75 ml. of blood. Fifteen days postinjection.)



The gross lesions were similar to those of the left femoro-tibial joint at three months postinjection (Figure 20). The more severe changes consisted of erosions of the articular surface and vascularization of the tangential zone with metaplasia to fibrous tissue. Beneath the metaplastic area, the chondrocytes were irregularly arranged and had undergone degenerative alterations of pyknosis or karyolysis. Many lacunae contained only cellular debris.

Focal hyperplasia of the chondrocytes in the tangential zone of one animal occurred at the marginal area of the cartilage (Figures 26 and 27). These cells were stellate and had densely basophilic nuclei. In the adjacent germinal zone, was a localized area of degenerated chondrocytes in which the matrix stained unevenly basophilic (Figures 26 and 28). This area was associated with a reduction of metachromasia.

Fissures and extensive erosions (Figures 29 and 30) which involved the germinal zone were noted and were associated with a loss of metachromasia (Figure 31). The chondrocytes in and underlying these areas had degenerative changes of pyknosis, karyorrhexis and cytoplasmolysis. The matrix changes were associated with a loss of metachromasia.

Figure 24. Top. Vascularization of the tangential zone. Note the perivascular connective tissue. Hematoxylin and eosin stain. X 80.

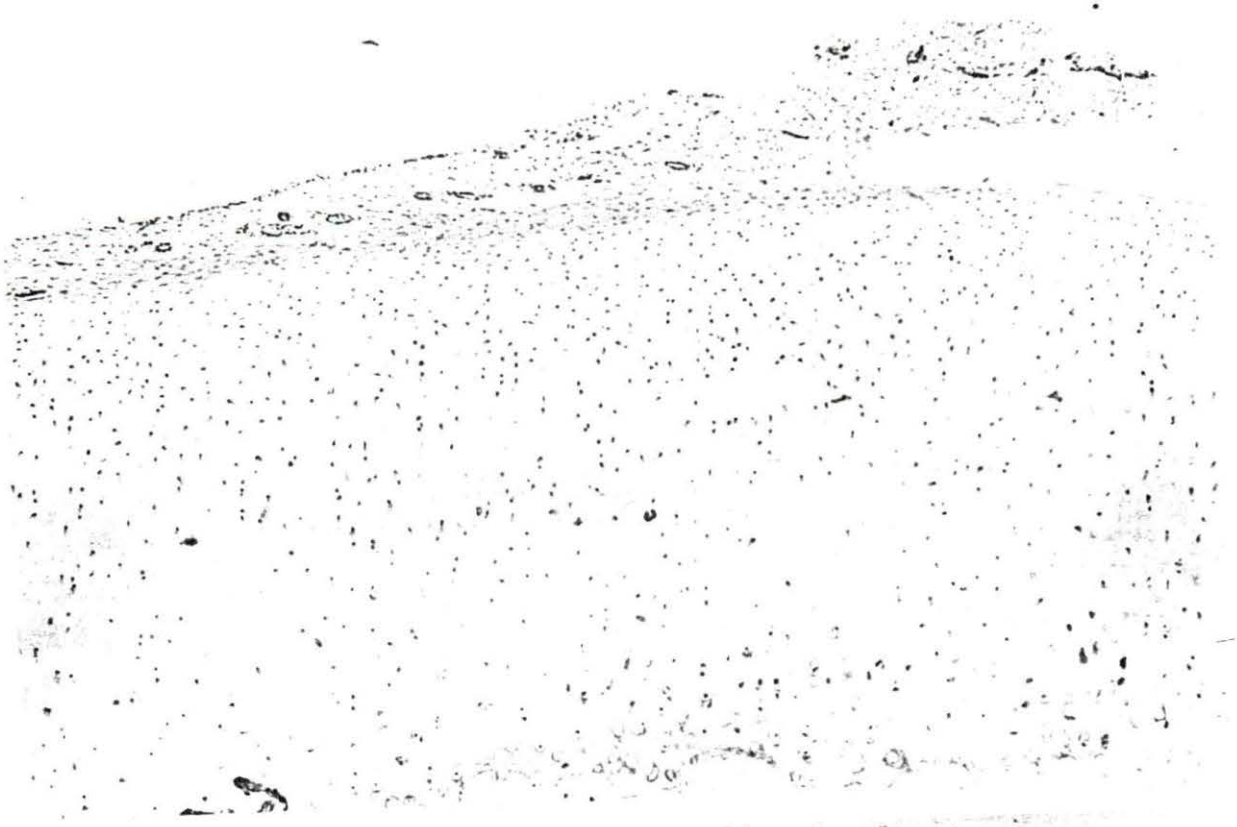
(Animal #175. Left femoro-tibial joint; injected with 75 ml. of blood. Three months postinjection.)

Figure 25. Lower left. Higher magnification of Figure 24. Note the irregular arrangement, pyknosis and cytoplasmolysis of the chondrocytes. Hematoxylin and eosin stain. X 230.

(Animal #175. Left femoro-tibial joint; injected with 75 ml. of blood. Three months postinjection.)

Figure 26. Lower right. Focal hyperplasia (A) of the chondrocytes of the tangential zone and focal area of basophilia (B) in the germinal zone. Hematoxylin and eosin stain. X 55.

(Animal #176. Right femoro-tibial joint; injected with five ml. of blood. Three months postinjection.)



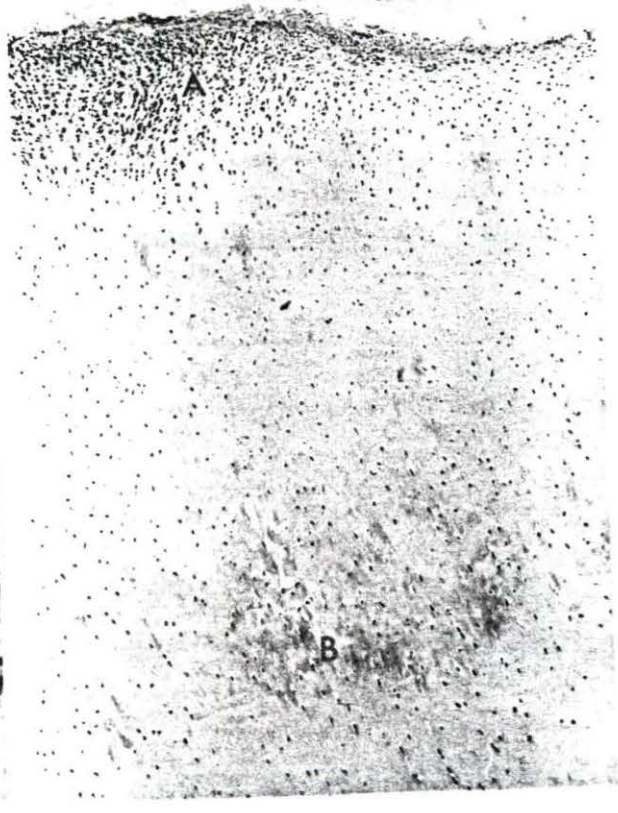

A
B

Figure 27. Higher magnification of the cartilage surface in Figure 26. Note the pyknotic, stellate, irregularly oriented chondrocytes. Hematoxylin and eosin stain. X 190.

(Animal #176. Right femoro-tibial joint; injected with five ml. of blood. Three months postinjection.)

Figure 28. Higher magnification of the focal basophilic area in Figure 26. Note the pyknosis and cytoplasmolysis of the chondrocytes. Hematoxylin and eosin stain. X 190.

(Animal #176. Right femoro-tibial joint; injected with five ml. of blood. Three months postinjection.)

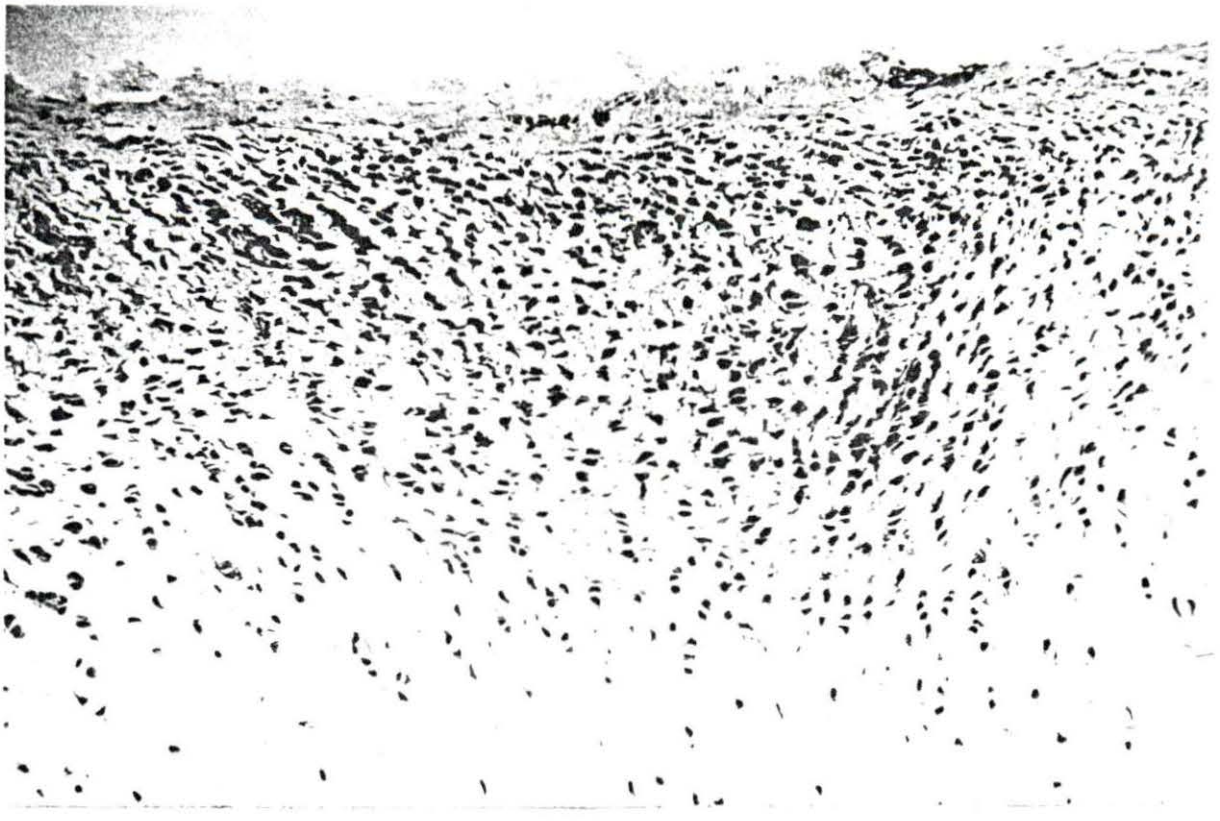


Figure 29. Extensive erosion of the articular cartilage. Hematoxylin and eosin stain. X 115.

(Animal #176. Right femoro-tibial joint; injected with five ml. of blood. Three months postinjection.)

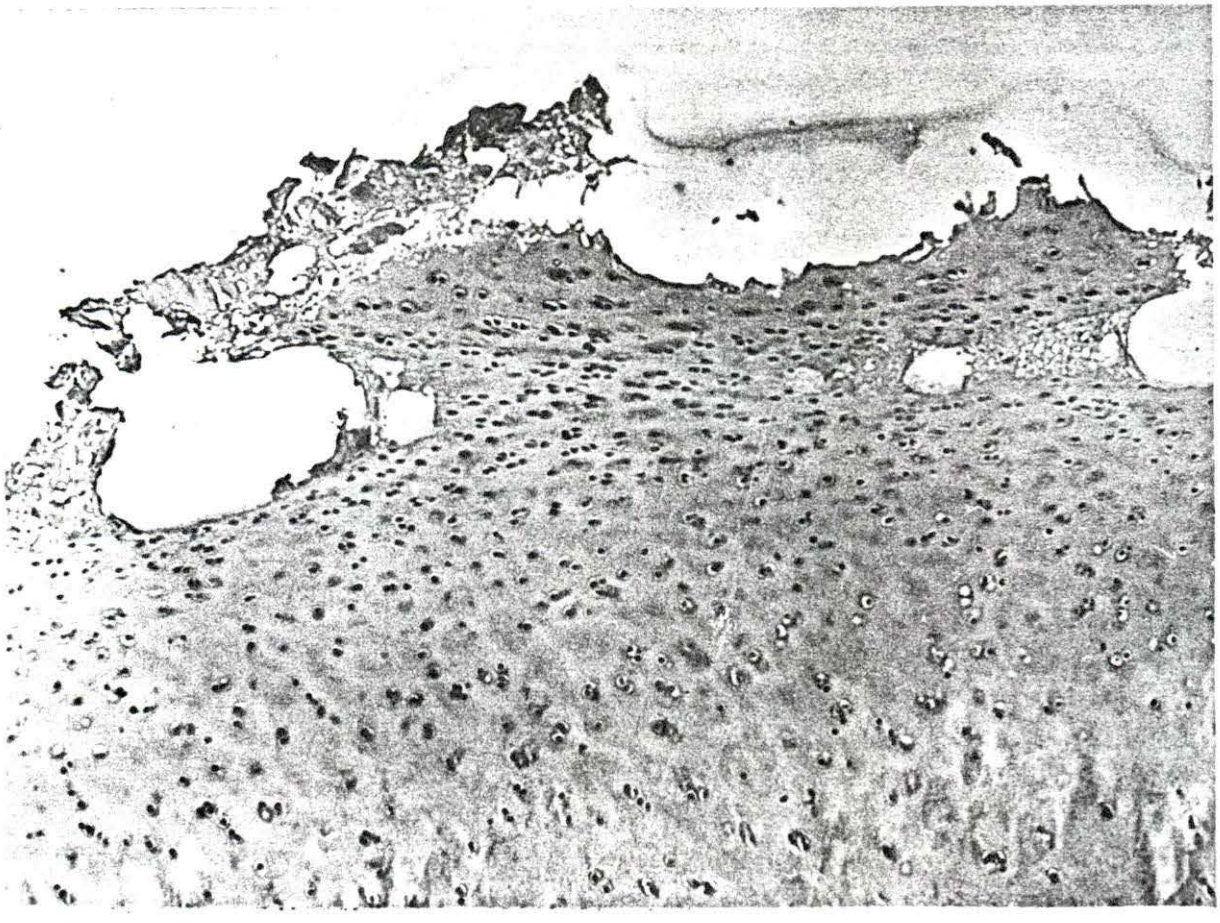
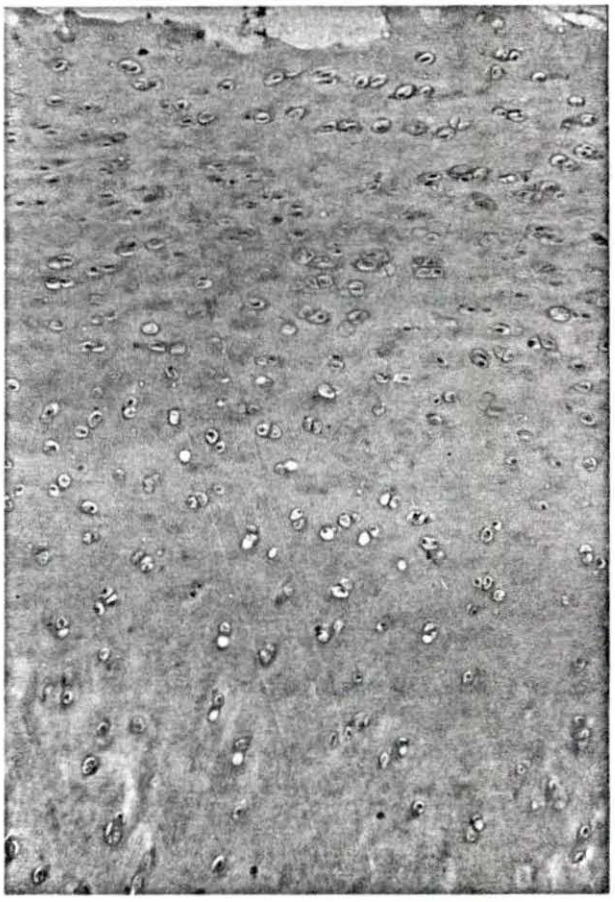


Figure 30. Higher magnification of erosion in Figure 29. Note the pyknosis and cytoplasmolysis of the chondrocytes. Hematoxylin and eosin stain. X 350.

(Animal #176. Right femoro-tibial joint; injected with five ml. of blood. Three months post-injection.)

Figure 31. Loss of metachromasia at the surface of the erosion shown in Figures 29 and 30. Toluidine blue stain. X 60.

(Animal #176. Right femoro-tibial joint; injected with five ml. of blood. Three months post-injection.)



DISCUSSION

The term degenerative osteoarthritis as related to diarthrodial joints is commonly associated with the following changes: fibrillation, fissuring, erosion, osteophyte formation at the margins of the articulations and sub-chondral cyst formation (Abrams, 1960; Bennett, 1956; Callender and Kelser, 1938; Johnson, 1959; Jubb and Kennedy, 1963). Sub-chondral cyst formation is a characteristic which is most prominent in man; however, it has been reported in domestic animals (Shupe, 1959).

According to some authors, the practice of training and racing young and immature horses has greatly increased the incidence of lameness problems in the equine (Vickers, 1962; Mackay-Smith, 1962) and "has generated an inexhaustible supply of horses otherwise apparently perfectly healthy, with every degree of osteoarthritis initiated by excessive exercise" (Mackay-Smith, 1962).

Synovial Membrane

Investigators of equine degenerative arthritis (Hare, 1927; Callender and Kelser, 1938; and Mackay-Smith, 1962) have noted hypertrophy of the synovial villi in arthritic joints. This is more common in equine than in human degenerative osteoarthritis (Sokoloff, 1960). Experimentally, hemarthrosis has been incriminated as an etiologic factor of hypertrophy of the

synovial villi (Key, 1929; Young and Hudacek, 1954).

A single injection of blood into equine joints did not produce marked alterations in the synovial membrane in the present study. However, the lesions observed were most prominent in those joints which were filled with blood. The changes in the synovial membrane were most marked 15 days and one month postinjection, and were more severe in the two joints that had been filled to maximum capacity with blood than in the joint which received five ml. of blood. These alterations were less severe at two months postinjection and by three months postinjection they had nearly disappeared.

The gross changes in the synovial membrane consisted of congestion and yellow to reddish-yellow discoloration. The microscopic alterations observed consisted of enlargement and hypochromasia of the nuclei of the synovial cells, the presence in the synovial membrane of an increased number of macrophages, perivascular cuffing in the synovial membrane by lymphocytes and plasma cells, infiltration of the synovial membrane by foam-type macrophages, and hemosiderin within macrophages and synovial cells.

The Turnbull's blue staining reaction, which is specific for free iron, was used to determine if the pigment noted in the hematoxylin and eosin stained sections was hemosiderin. Hemosiderin was noted in the synovial membrane in amounts directly related to the amount of blood injected and for a period of time which was inversely related to the time since

injection of the blood. It had been phagocytized by macrophages and synovial cells but the hemosiderin was not engulfed by the lymphocytes and plasma cells which infiltrated the tissue in the perivascular areas. Cartilage sections stained by this procedure failed to show evidence of hemosiderin within the cartilage.

Enlargement of the nuclei of the synovial cells was also observed by Key as a response to experimental hemarthrosis in rabbits (1929) and in human hemophilic arthritis (1932). However, in the latter report, he stated that these nuclei stained densely basophilic. This nuclear staining reaction is different from that found in the current work. Recently, Ball et al. (1964), using the electron microscope, observed enlargement of the synovial cells in joints which had received intra-articular injections of iron dextran, but this was apparently due to an increase in cytoplasmic size. In the present study, cytoplasmic size was not evaluated as the limits of the cytoplasm of normal synovial cells are not well defined with the light microscope.

Perivascular cuffing by lymphocytes and plasma cells in the synovial villi, as well as the presence of macrophages which contain hemosiderin, both of which were found in this experiment, have also been noted in pigmented villonodular synovitis (Jaffe et al., 1941; Greenfield and Wallace, 1950; Shafer and Larmon, 1951; Jaffe, 1958; and Robbins, 1962), hemophilic arthritis (Key, 1932; Swanton, 1957 and 1959;

Rodnan, 1959 and 1960) and in experimental hemarthrosis (Key, 1929; Young and Hudacek, 1954). Various authors have confirmed the fact that synovial cells are involved in phagocytosis. Key (1929) observed that after intraarticular injection of india ink into the femoro-tibial joints of rabbits, a small amount of fine carbon was taken up by the synovial cells. Young and Hudacek (1954) observed material, which stained positively with an iron stain, in the synovial cells of joints which had received repeated injections of blood. Ball et al. (1964), in a recent electron microscopic study, have confirmed the ability of the synovial cells to phagocytize iron-containing material.

Foam-type macrophages have been noted in pigmented villonodular synovitis (Jaffe et al., 1941; Greenfield and Wallace, 1950; Shafer and Larmon, 1951; Jaffe, 1958; and Robbins, 1962) and in experimental hemarthrosis (Young and Hudacek, 1954).

The fact that all the above alterations have previously been observed in villonodular synovitis, experimental hemarthrosis and hemophilic arthritis as well as in the present research demonstrates that the synovial membrane changes herein reported were due to the injected blood. These findings also suggest that the overall response of the synovial membrane was inflammatory in nature.

Swanton (1957), in regard to hemophilic arthritis, noted that "lesions with all the gross and microscopic characteristics of pigmented villo-nodular synovitis have not been seen.

Hemosiderin pigmentation, with abundant macrophages, and moderate hyperplasia, fibrosis, and sometimes adhesions of synovial villi do occur, but large arborescent and nodular villous masses have not been present in the many observed stages of reaction to repeated hemarthrosis in these hemophilic dogs. The giant cells, foam cells, and sheets of actively proliferating synovial stromal cells seen histologically in pigmented villo-nodular synovitis are not a significant feature of the lesions in these animals."

The changes which Swanton claimed were not present in hemophilic arthritis were likewise not noted in the current study. Thus, the synovial membrane lesions in the latter work are more closely associated with hemophilic arthritis, in which blood is surely the primary etiologic agent, than with villo-nodular synovitis in which blood is not undisputably the underlying etiologic factor (Jaffe et al., 1941; and Jaffe, 1958).

The nuclear changes of the synovial cells and phagocytosis of hemosiderin by synovial cells were the most frequent alterations noted in the synovial membrane in the present study. Furthermore, these nuclear changes and the amount of hemosiderin noted in each injected joint at each postinjection time period indicated that the degree of change in the synovial membrane had a direct relationship with the amount of blood injected and, after one month postinjection, had an inverse relationship with the postinjection time. The absence of hypertrophy of the synovial villi suggests that repeated

episodes of hemarthrosis are necessary for the responses reported with villonodular synovitis, hemophilic arthritis and experimental hemarthrosis.

Articular Cartilage

Previous findings in the literature suggest that intra-articular hemorrhage may produce or be involved in the pathogenesis of degenerative osteoarthritis in the horse. First, Young and Hudacek's (1954) and Robbins' (1962) incrimination of blood as a cause of villonodular synovitis offers an association between blood and equine degenerative osteoarthritis as, in the latter, the villi are also often hypertrophic (Sokoloff, 1960). Secondly, observations on hemophilic humans (Keefer and Myers, 1933) and hemophilic canines (Swanton, 1959) have shown that in joints with evidence of previous hemorrhage, the articular cartilage often had changes which were typical of the type occurring in degenerative osteoarthritis. Thirdly, intra-articular hemorrhage has been listed among the possible causes of degenerative osteoarthritis in both man and the horse (Keefer et al., 1934; Sippel, 1942a; and Sokoloff, 1960).

The effects of blood in the equine diarthrodial joint have not previously been reported. Furthermore, in those experiments in which blood had been injected several times into diarthrodial joints in smaller laboratory animals (Key, 1929; and Young and Hudacek, 1954), no significant cartilage defects were noted.

In the present study, however, both gross and microscopic lesions were observed. The initial gross alteration of the articular cartilage was a dull white discoloration. This was followed by focal reddish discoloration. The next stage in the progression of the changes was a more generalized yellowish-red mottling over the dull white cartilage. In the left radio-carpal joints, which were filled with blood, these reddish areas were usually seen near the anterior margin of the articular cartilage and were accompanied by softening and roughening of the cartilage. In the femoro-tibial joints, regardless of the amount of blood injected, reddening, softening and roughening of the cartilage first appeared in the central one fourth of the articular surfaces of the distal end of the femur and the corresponding opposing surfaces of the proximal end of the tibia. By three months postinjection, the central one half of the articular surfaces was involved. Grossly observable erosions were present by two months and fissures at three months postinjection. Keefer et al. (1934) examined 100 unselected knee joints of man and made similar observations on the location of the cartilage lesions in the femoro-tibial joints. He noted that in about one half of these there was erosion or marked thinning of the articular cartilage of the distal end of the femur and the proximal end of the tibia. This was most conspicuous at the point where the condyles of the femur were in contact with the central portion of the tibia

uncovered by the lateral and medial menisci. The location of the lesion coincides with the weight bearing portion of the articular cartilages. This finding suggests that the motion and pressure of weight are factors in the development of the lesion.

Swanton (1959), in her observations on hemophilic dogs, noted that in mild cases the articular cartilage was dull, granular, velvety and soft. Key (1932) noted that in addition to the marginal destruction, there was a variable amount of focal destruction of the articular surfaces which were irregular in contour and "map-like" in character. He believed this to be characteristic of the hemophilic joint. These reports seem to describe gross alterations similar to those seen in the current study.

Abrams (1960) stated that the first gross changes of degenerative joint disease were softening, roughening and fibrillation of the articular cartilage. He said that definite "crevasses" appeared in the cartilage along with the appearance of clefts and pits. The "crevasses" were probably the same change which was noted in the three month postinjection joint and which has been herein described as grossly observable fissuring.

The first microscopic change noted was chondrocyte degeneration. This was first found 15 days postinjection in the cartilage of the right femoro-tibial joint but was present at

one, two and three months postinjection in all joints which were injected with blood.

A focal granularity of the matrix and an intensely eosinophilic staining matrix were usually found concurrently and were usually associated with degenerated chondrocytes. These matrix changes were found in all the joints which were injected with blood of all animals killed one, two and three months postinjection. These alterations apparently followed the cellular degeneration.

When noted, basophilic stippling of the matrix was associated with degenerating chondrocytes. However, this change was noted only in the left and right femoro-tibial articular cartilages of one animal. This was a manifestation of chondrocyte degeneration but was a less frequent finding than cytoplasmolysis, chromatolysis, pyknosis and karyorrhexis.

An irregular distribution of chondrocytes with a disruption of their columnar orientation was present in the left femoro-tibial joints at one, two and three months postinjection and in the right femoro-tibial joints in all animals examined. This change was not noted in any of the left radio-carpal cartilages.

The literature on degenerative osteoarthritis and hemophilic arthritis has only superficially described the cellular and matrix changes of the cartilage. The granular, more intensely eosinophilic matrix found in this study has not been

reported in the literature reviewed. It is probable that this is because previous reports have dealt with cartilage that had been undergoing degeneration for a time longer than the duration of the present experiment. It is felt that the alterations herein described were earlier changes in the degenerative processes than those previously reported.

Fibrillation and erosion of the articular surface were inconsistent findings as late as two months postinjection. However, in the three month postinjection animals, all the cartilages of all the joints into which blood was injected had these changes.

Fissures were present only in the femoro-tibial articulations. These occurred as early as 15 days in the left femoro-tibial joint. They were not noted at one month, but appeared in the right femoro-tibial articular cartilages at two months postinjection. At three months postinjection, fissures were present in the left femoro-tibial joints of both animals but were present in the right femoro-tibial joint only in animal 176.

Microscopic fibrillation, erosion and fissuring of the articular cartilage has been frequently described in degenerative osteoarthritis and other conditions of the joint in which blood has been incriminated as an etiologic agent. Key (1929) observed, in rabbits whose joints were injected with blood seven times, that the surface of the cartilage was usually

normal, but in some areas it was roughened and frayed. Breimer and Freiberger (1958) noted fibrillation and erosion of the articular cartilage associated with villonodular synovitis. Swanton (1959) stated that the more severe changes found in hemophilic arthritis were pitting to focal gray or reddish eroded areas of extensive coarse roughening of the surface. Hare (1927) described cleavage of the cartilage matrix in equine arthritis which is probably similar to that which is now referred to as fibrillation. In the series of unselected equine joints which Sippel (1942a) examined, erosion was the most common gross lesion. Bennett (1957) also described fibrillation and erosion in degenerative osteoarthritis of man.

Vascularization of the tangential zone with metaplasia of the hyaline cartilage to fibrous tissue or fibrocartilage was seen in five of the six joints examined at three months post-injection. The joint not showing this change was one of the two left radio-carpals. This lesion has not been described in degenerative or hemophilic arthritis. Key (1929) stated that in rabbit joints in which blood had been injected seven times, the chondrocytes were not enlarged and showed no proliferation except near the marginal area of the joint where the reaction in the synovial membrane tended to involve the adjacent cartilage for a short distance. This vascularization and metaplasia found in the current experiment probably accounts for the focal reddening and some of the softening noted at necropsy.

However, these areas were not the result of pannus formation.

Key (1932) felt that the cartilage damage in hemophilic joints was due to pannus formation over the articular cartilage with subsequent depletion of nutrition to the cartilage. In regard to the lesions at the margin of the articular cartilage, Rodnan (1960) agreed. However, he did not think that the lesions in the central part of the surfaces were caused by a pannus. Swanton (1959) did not attribute the genesis of the articular changes of the hemophilic joint to pannus formation from the hyperplastic synovial villi. She stated that "organization of fibrin on articular cartilage surfaces to form a pannus or intercartilagenous adhesions has not occurred."

Pannus was discounted as an explanation for the metaplasia of the surface in this research because (1) there was minimal involvement of the synovial membrane at the time when the metaplasia was observed, (2) no pannus was observed grossly, and (3) even where metaplasia occurred at the marginal area of the joint (Figure 16), microscopic examination revealed no connection between the marginal areas and the metaplastic areas in the central part of the joint.

Focal hyperplasia of the chondrocytes of the tangential and outer germinal zones with underlying focal degeneration of the matrix was seen in only one joint (right femoro-tibial). This occurred at three months postinjection. The significance of this change is not definitely known. Hare (1927) noted that in equine arthritis, the cartilage cells near the surface

seemed to multiply and appear in clones of four to eight cells. In this experiment, the focal hyperplastic area was composed of cells which were stellate, spindle-shaped and pyknotic but were present in far greater numbers than Hare's description indicates. This change has not previously been described in the literature and is difficult to evaluate. However, it may be an inceptive stage of degenerative osteoarthritis.

Some of the changes noted in the current study were not present in the left radio-carpal cartilages, or at least were less severe than in either of the femoro-tibial joints. This suggests that the differences in type of motion between the radio-carpal and femoro-tibial joint are a more significant factor than weight alone in the pathogenesis of the observed lesions.

Slight differences in the occurrence and severity of lesions existed between the right and left femoro-tibial joints. These, however, were not consistent. The severity and occurrence between these joints was often reversed at different postinjection times or between the two animals at the same postinjection time. It is apparent that the amount of blood injected is not significant in the production of the observed degenerative cartilage lesions.

In this study, loss of metachromatic staining of the cartilage matrix was associated with: (1) degenerated chondrocytes, especially where the matrix was granular and very eosinophilic, (2) fibrillation and erosion, especially at the

surface, and (3) the matrix at the margins of fissures.

Single-dyes that stain various tissue components a different color than the original dye are said to be metachromatic (Davenport, 1960). Orthochromatic staining denotes a tissue color similar to the color of the dye (McManus and Mowry, 1960). Toluidine blue is a metachromatic dye which stains normal cartilage matrix violet to red (Bélanger and Hartnet, 1960). Metachromatic staining of hyaline cartilage depends upon the presence of sulphate groups on the chondroitin sulphate molecule of the ground substance (Sylvén, 1956; Szent-Györgyi, 1960). Matthews (1953) conducted chemical analyses on the fibrillated cartilage found in degenerative arthritis and on healthy cartilage from the same joints. He found that in degenerative arthritis, the ground substance was lost at a faster rate than the collagen and that the collagen/chondroitin sulphate ratio increases in this condition. In degenerative arthritis, loss of chondroitin sulphate should be reflected by a loss of metachromasia with toluidine blue staining. In the current study, most areas of matrix degeneration stain orthochromatically suggesting a loss of chondroitin sulphate.

Loss of metachromasia was not consistently found in association with all lesions observed with hematoxylin and eosin staining, however. An explanation for this is very difficult. Conversely, orthochromatic staining was observed in a few areas in which no changes were detected with hematoxylin and

eosin stain. Possible explanations for this inconsistency have been mentioned by Sylvén (1956): (1) sulphate, and hence chondroitin sulphate, is present but linked in such a way that the surface charge density has decreased below a certain minimum level necessary for linkage with the dye, (2) the concentration of the sulphate groups available (at the surface) to the dye is below a "threshold value" necessary for metachromatic staining.

The fact that no clinical evidence of lameness was present is not inconsistent with the previous findings on degenerative osteoarthritis both in man and in the horse (Keefer et al., 1934; Callender and Kelser, 1938; Sippel, 1942a; Bennett, 1957; Abrams, 1960; and Sokoloff, 1960).

Many explanations of the pathogenesis of degenerative arthritis have been given for both man and horse. Probably the most commonly accepted and most plausible current theory is the concept that degenerative osteoarthritis in both species is a "normal" condition of aging. It occurs in almost all individuals as they approach senility, and the incidence of the condition increases with age. As the individual grows older, the morphology of the joint changes by differential rates of growth and by progressive and regressive remodeling and by peripheral circumferential expansion. As long as these mechanisms are balanced, there is no abnormality of structure or function and no disease (Johnson, 1962). As early as 1934, Keefer et al. concluded that "degenerative arthritis is a

process associated with the aging of the tissues of the joints, aggravated by strain, hemorrhage, trauma, and static deformities, the end results depending on a summation of these factors." However, where the condition occurs in the young individual prior to a time in which the condition would be considered "normal", it is considered pathologic (Jubb and Kennedy, 1963). During life, the joints may be subjected to insults which produce degenerative osteoarthritis via unbalanced remodeling. Hence, "pathologic" degenerative osteoarthritis is produced in the young animal.

Johnson (1962) gave a list of initiating factors which lead to unbalanced remodeling. These included trauma which shears off and fragments deep layers of cartilage, and extensive change in the osmotic properties of the synovial fluid which inhibit diffusion nutrition of the cartilage (as from repeated hemarthrosis in hemophilia). Degenerative effects upon the articular cartilage produced by an altered physical-chemistry depend on the repeated nature of the hemarthrosis of hemophilia. In the present study, another mechanism must be involved since blood was injected only once and since the lesions continued to increase in severity throughout the experiment. Some component or components of the injected blood initiated changes in the articular cartilage which were probably continued by the motion and weight upon the articular cartilages. It is postulated that the blood hastened the process of the

development of "normal" degenerative osteoarthritis and produced an early onset of the condition, which because of the young age of the animal, would be classified as "pathologic".

The results of this study indicate that saline or serum did not produce alterations in the articular cartilage. It is therefore apparent that some cellular component of blood is responsible for these lesions. Furthermore, it has been demonstrated that the quantity of blood is not the significant factor.

The most severe lesions in this study were similar to those which have been described for degenerative osteoarthritis. The degenerated chondrocytes surrounded by a granular, more intensely eosinophilic matrix, as well as the loss of columnar orientation of the chondrocytes in the germinal zone, may correspond to the early changes of degenerative osteoarthritis. Blood did produce such changes in the equine diarthrodial joint whereas serum alone or saline did not initiate changes. This, together with the fact that the lesions of degenerative osteoarthritis and hemophilic arthritis are almost indistinguishable, is felt to be circumstantial evidence that a cellular component of blood may be involved in the pathogenesis of degenerative osteoarthritis.

SUMMARY

1. Autogenous blood was injected into three diarthrodial joints of seven 18 to 24 month old Shetland ponies. The left radio-carpal and left femoro-tibial joints were filled to their maximum capacity (approximately 15 and 75 ml. respectively). The right femoro-tibial joint received five ml. of blood.

2. The right radio-carpal joint of every second animal was injected with 13 ml. of autogenous serum. This joint on alternate animals was filled with approximately 15 ml. of sterile physiologic saline and served as an injection control joint for each animal. These joints of an eighth pony were utilized as uninjected controls for the entire group.

3. The animals were markedly lame following injection. Following pasture exercise on the fourth day postinjection, no further signs of lameness were noticed. Lameness corresponded to the time that the joint capsule remained markedly distended.

4. One animal was killed at 15 days, two at one month, two at two months and two at three months postinjection.

5. The initial changes in the synovial membrane were observed at 15 days postinjection and consisted of enlargement and hypochromasia of the nuclei of the synovial cells and infiltration by macrophages. These cells contained phagocytized hemosiderin. These alterations were again observed at one month and to a lesser degree at two months postinjection.

Also, at these times, perivascular infiltration of lymphocytes and plasma cells which had not phagocytized hemosiderin was observed. The lesions were not present at three months. No alterations were present in the joints which received saline or serum or in the uninjected joints.

6. The synovial membrane changes were directly related to the amount of blood injected, not related to the type of joint injected, and were inversely related to the postinjection time. Villar hypertrophy was not a significant feature and the synovial membrane did not contribute to the cartilage lesions.

7. The initial gross change of the articular cartilage was a dull white discoloration which replaced the normal translucent bluish-white appearance. This was followed by focal reddish discoloration which, in turn, progressed to a more generalized yellowish-red mottling of the anterior edges of the left radio-carpal cartilages and the central portions of the cartilages on the condyles of the distal end of the femur and the proximal end of the tibia (involving both the left and right femoro-tibial joints).

8. The initial microscopic cartilage lesions at one month postinjection consisted of degenerative changes of the chondrocytes in the lower part of the tangential zone and upper part of the germinal zone. This was apparently followed by, but definitely associated with, a condensation of the matrix giving it a granular and more intensely eosinophilic

appearance in the area surrounding the degenerated chondrocyte. Two months postinjection there was reddening, softening and erosion of the articular surface; and microscopic lesions which consisted of fibrillation, erosion and disruption of the columnar orientation of the chondrocytes giving them an irregular arrangement.

The most advanced alterations noted in the three month group of animals consisted of: (1) mineralization of some areas of that part of the matrix which was associated with the surface of erosions, (2) vascularization of the tangential zone with metaplasia to fibrous tissue, (3) focal hyperplasia of the chondrocytes of the tangential zone.

9. Most of the lesions were associated with a decrease or loss of metachromasia when stained with toluidine blue.

10. The degree of cartilage change was directly related to the postinjection time, but was not related to the volume of blood injected. No changes were seen in the cartilage of joints which received serum or saline, nor in the uninjected joints.

11. The changes were more prominent in the femoro-tibial than in the radio-carpal joint.

12. This study demonstrates that autogenous whole blood will produce degenerative cartilage changes in equine diarthrodial joints and suggests that blood may be involved in the pathogenesis of degenerative osteoarthritis.

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